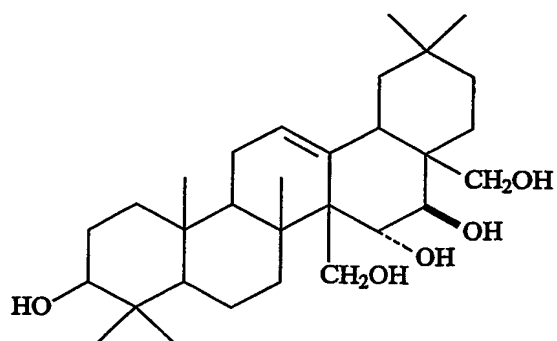
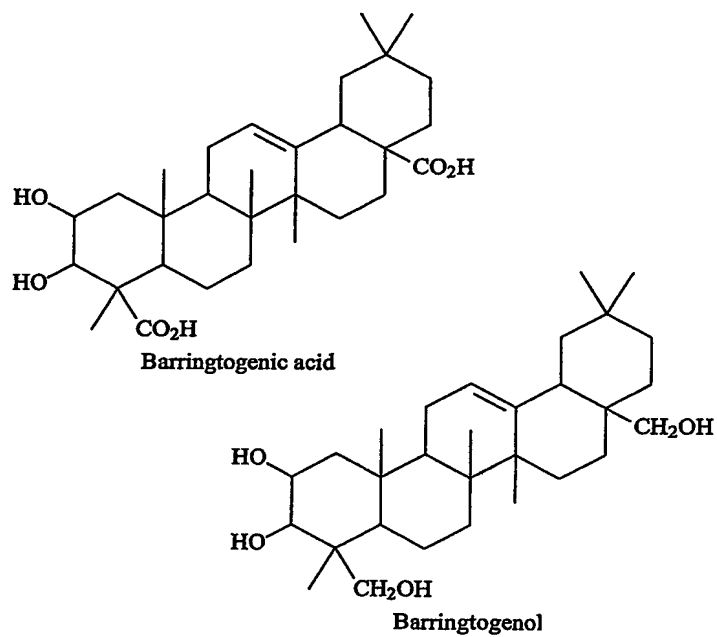
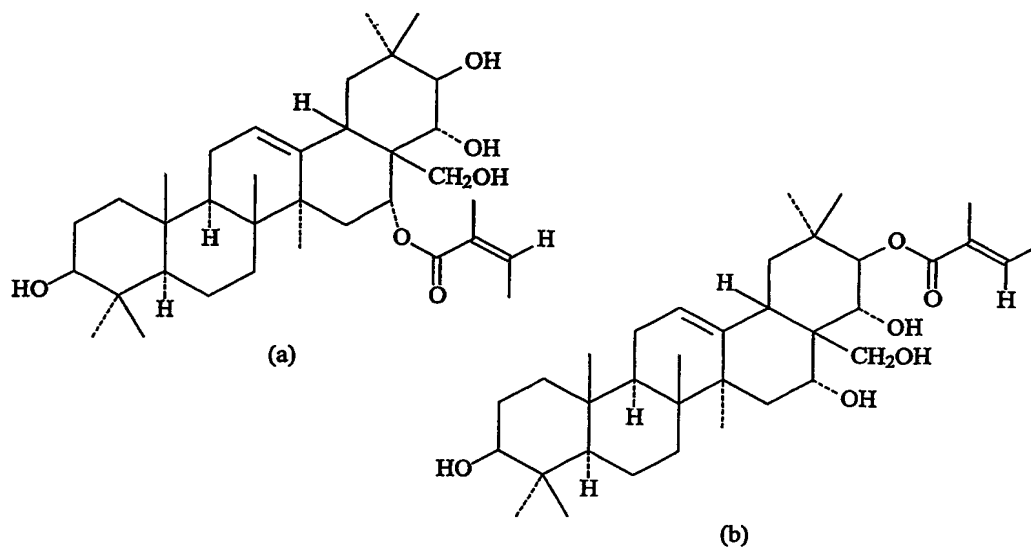
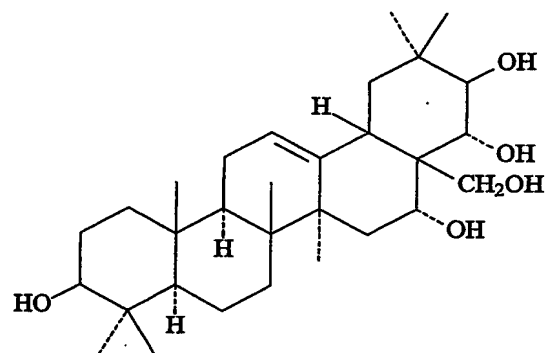
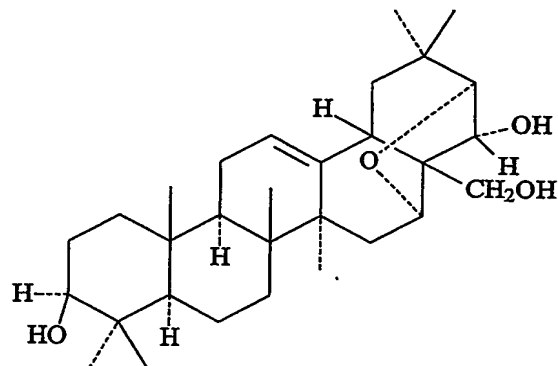


1/35

**FIG 1.** A₁-Barringenol**FIG 2.** The structure of barringtogenic acid and barringtogenol

2/35

**FIG 3 (a) Initial and (b) revised structures of barringtogenol B****FIG 4 Barringtogenol C****FIG 5 – Barringtogenol D**

3/35

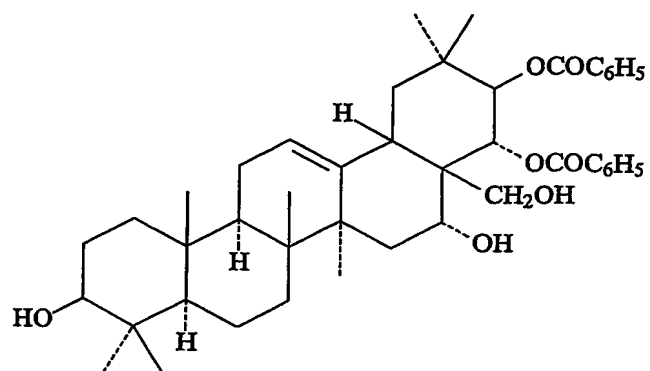
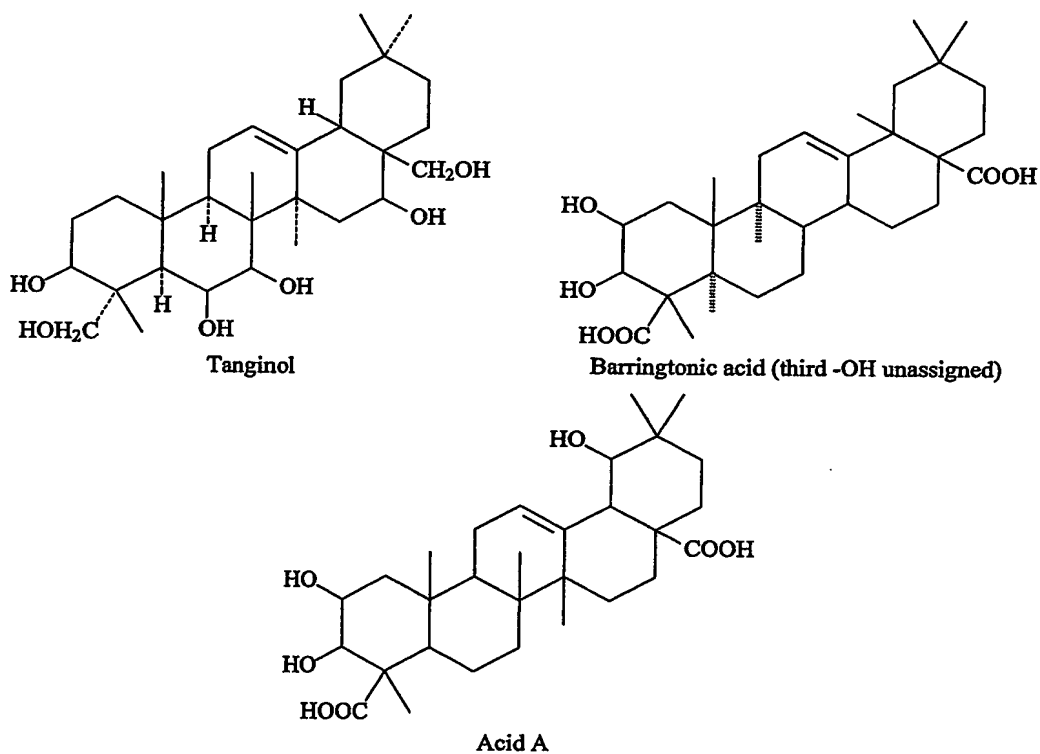


FIG 6 – Barringtogenol E

FIG 7 – Compounds from *B. acutangula*

4/35

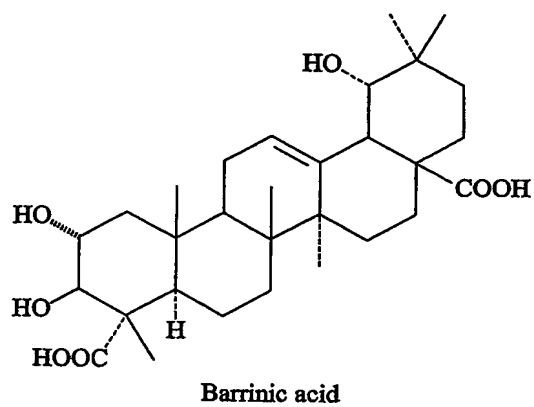
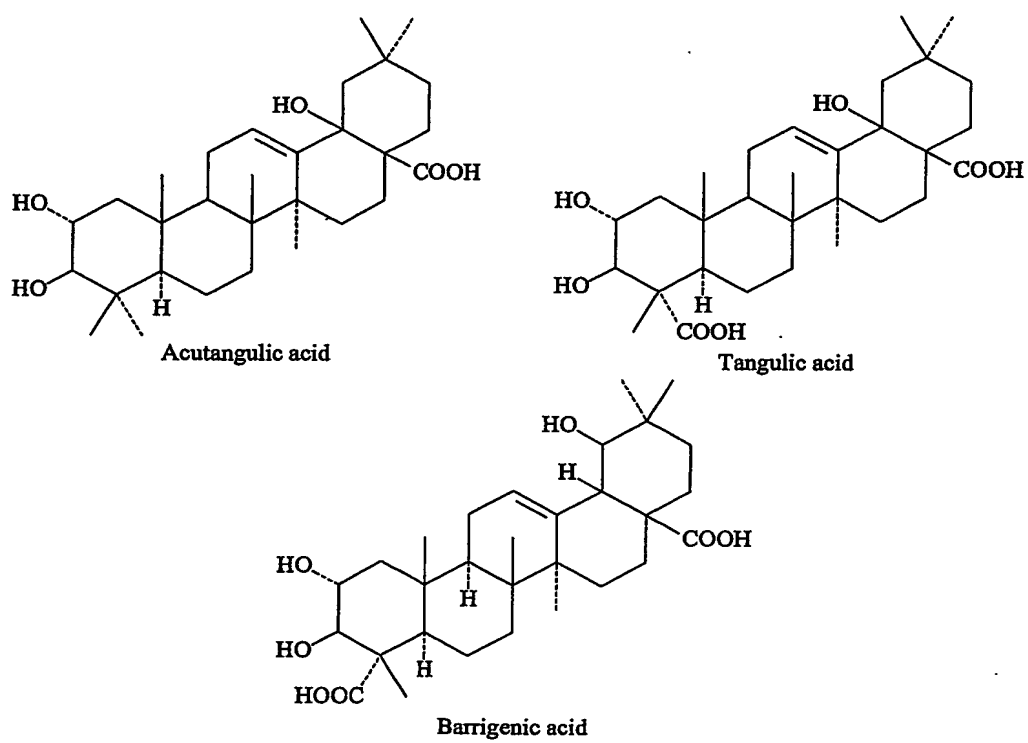


FIG 8. Barrinic acid

FIG 9 – Compounds from *B. acutangula*

5/35

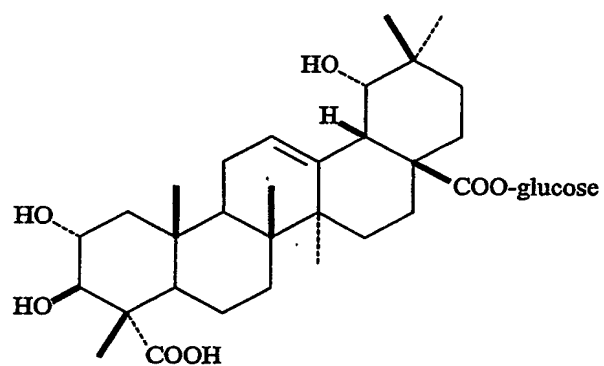
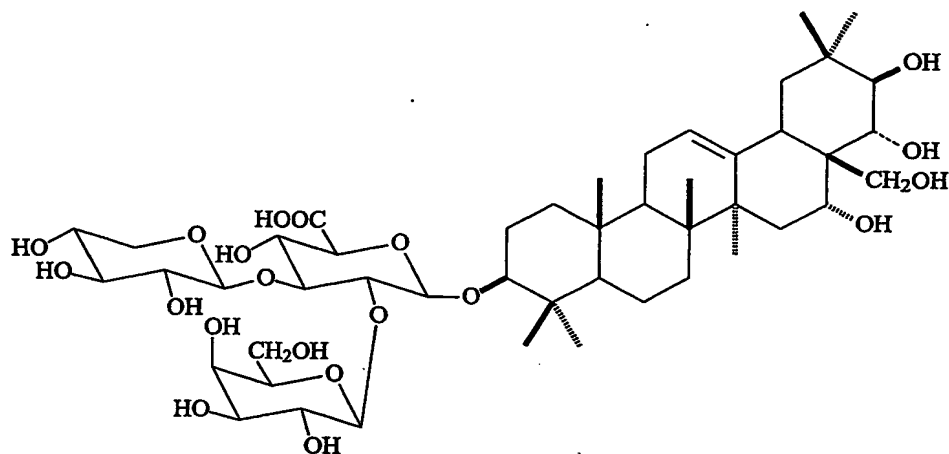
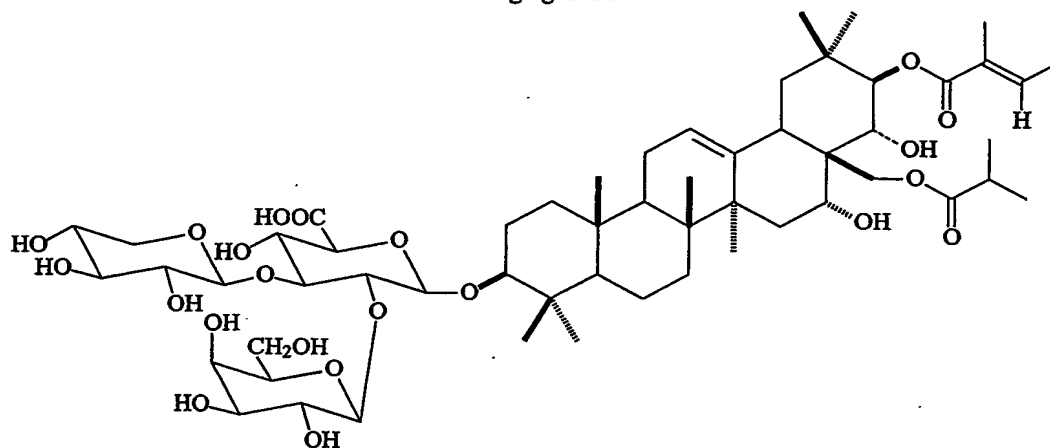


FIG 10 - 2 α ,3 β ,19 α -trihydroxy-olean-12-ene-dioic acid 28-O- β -D-glucopyranoside from the seeds of *B. acutangula*

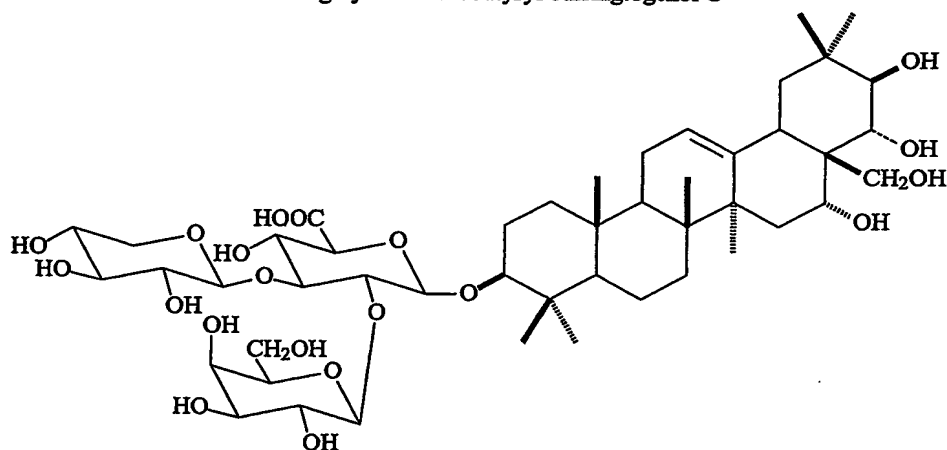
6/35



Barringoside A = 3-*O*- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl barringtogenol C



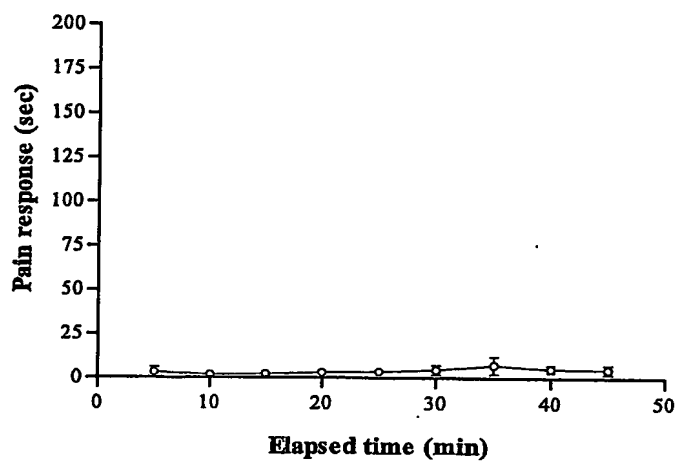
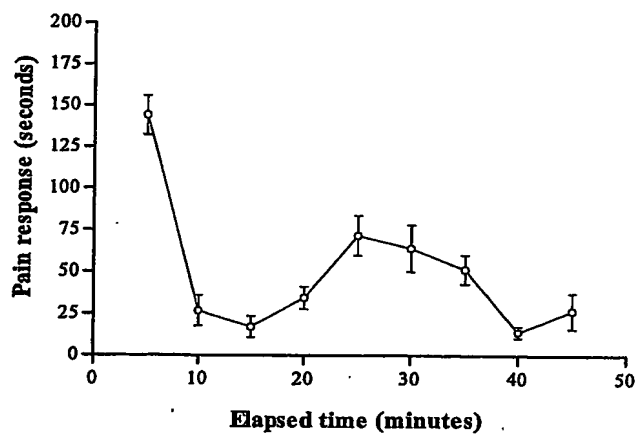
Barringoside B = 3-*O*- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl -21-*O*-tigloyl-28-*O*-isobutyryl barringtogenol C



Barringoside C = 3-*O*- α -L-arabinopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl barringtogenol C

FIG 11

7/35

**FIG 12 - Normal grooming response ($\bar{x} \pm \text{S.E.}$; $n = 6$).****FIG 13 - Control values ($\bar{x} \pm \text{S.E.}$; $n = 18$).**

8/35

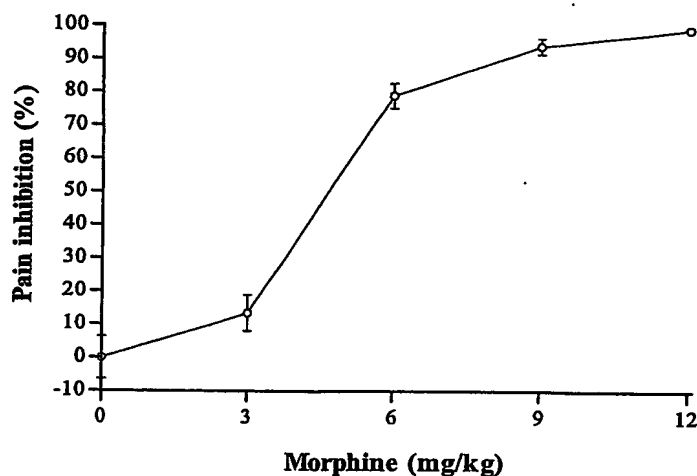


FIG 14 - Dose response curve for morphine ($\bar{x} \pm S.E.$; $n = 6(\text{min})$).

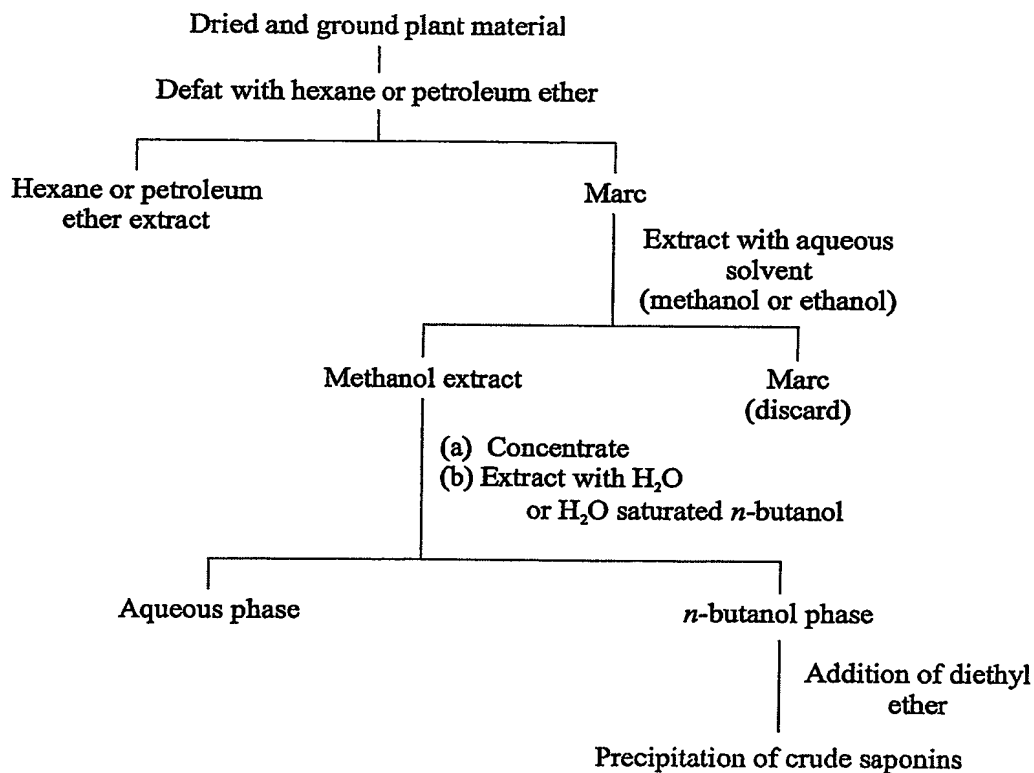


FIG 15 - Schematic for the preparation of crude saponin mixtures.

9/35

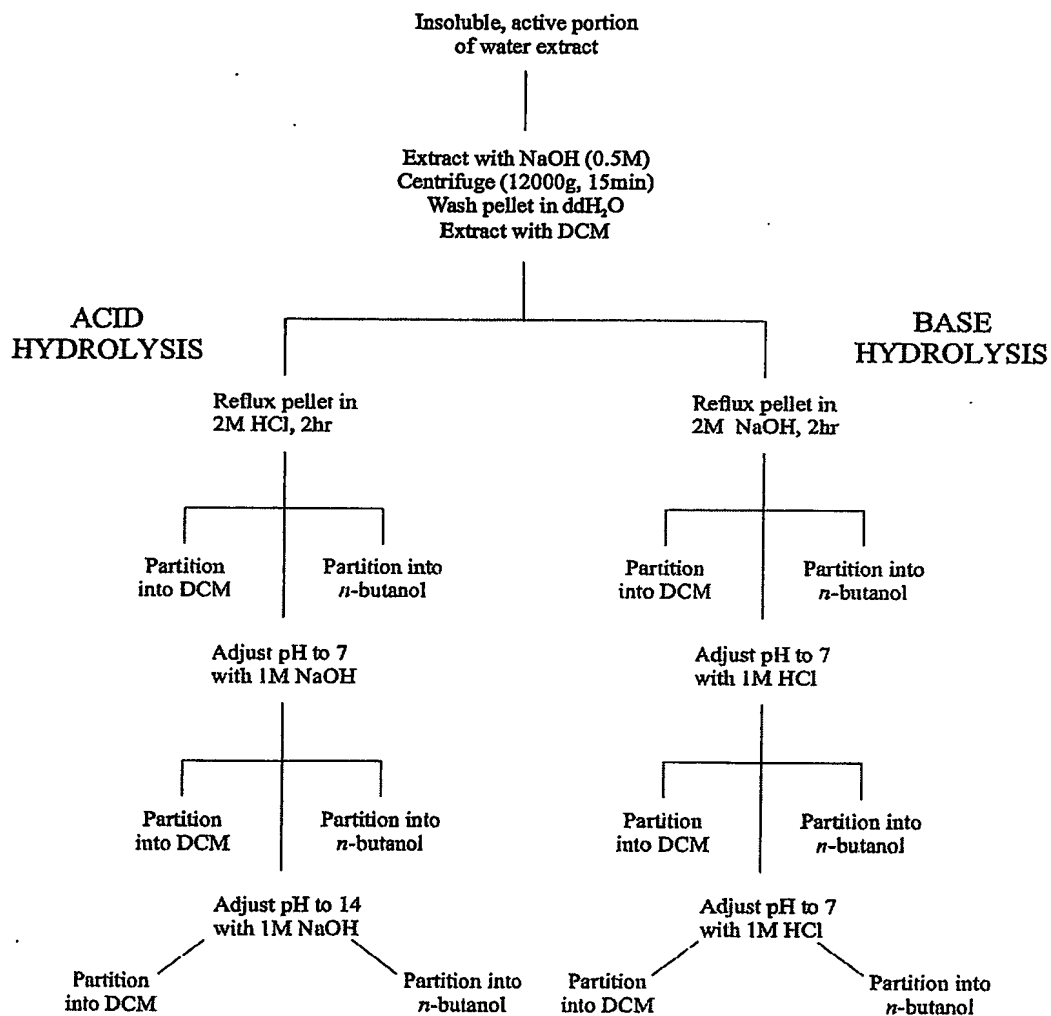


FIG 16 - Acid and base hydrolysis scheme.

10/35

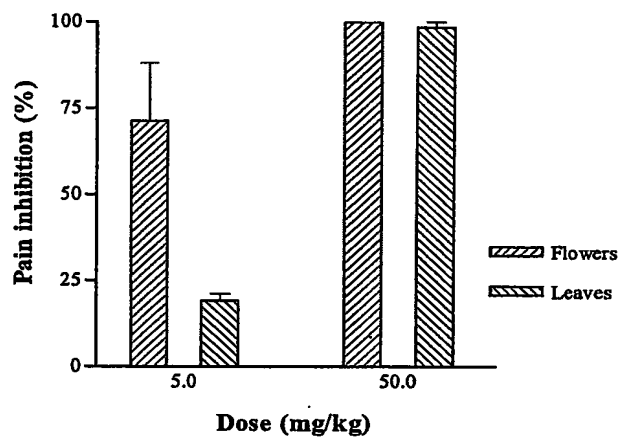


FIG 17 - Analgesic activity of water extract of flowers and leaves of *B. acutangula* ($\bar{x} \pm SE$, $n=2$).

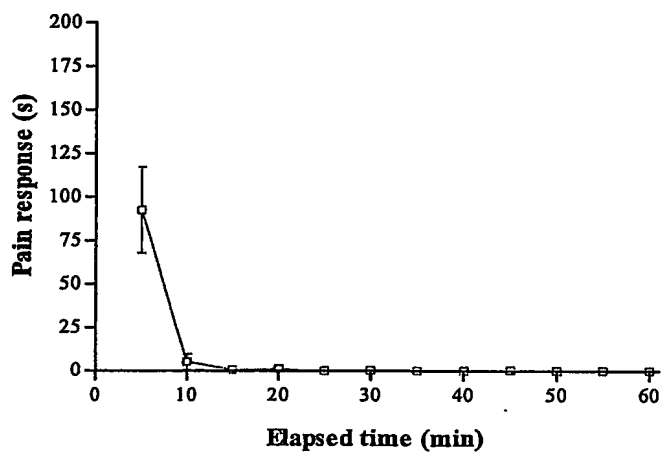


FIG 18 - Analgesic activity of crude water extract ($\bar{x} \pm SE$, $n=5$).

11/35

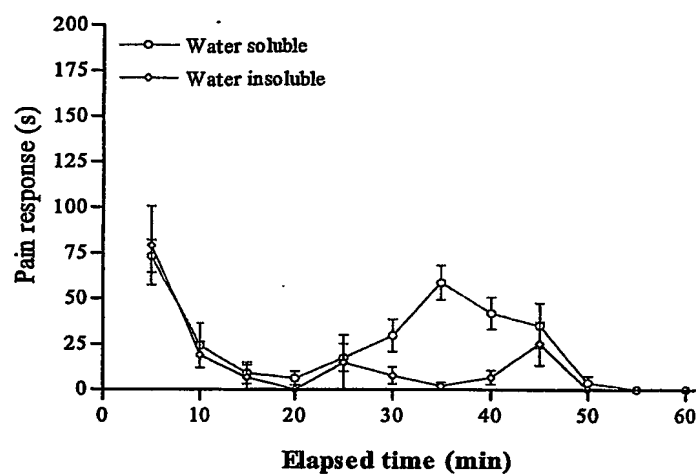


FIG 19 - Analgesic activity of crude water soluble (n=9) and insoluble (n=4) portions of the water extract ($\bar{x} \pm SE$).

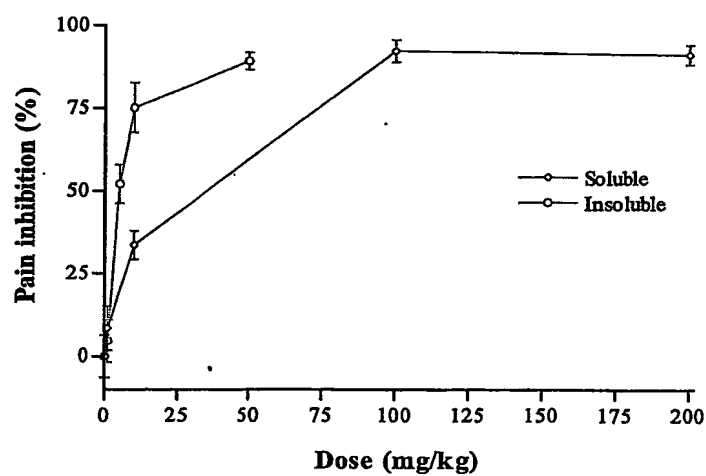
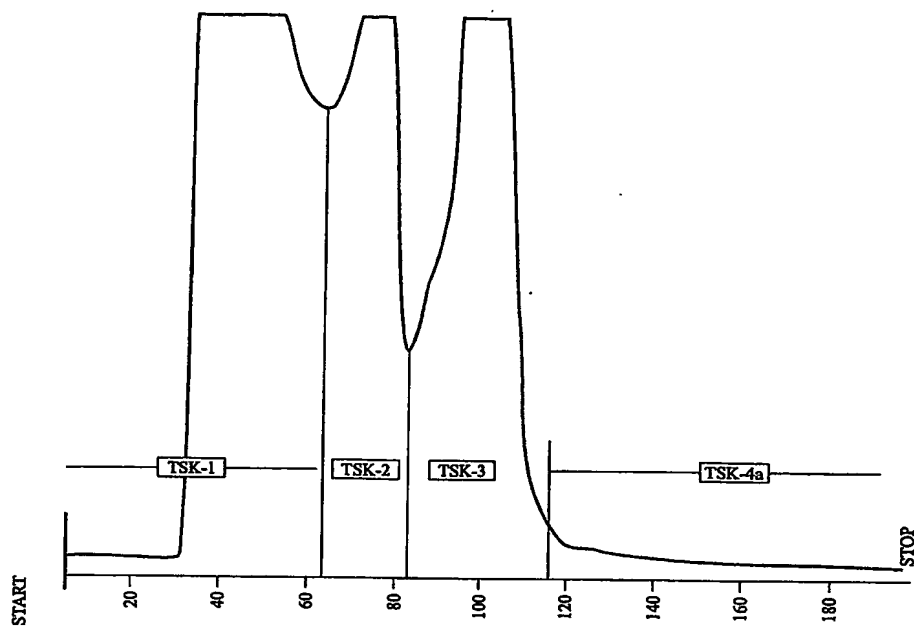
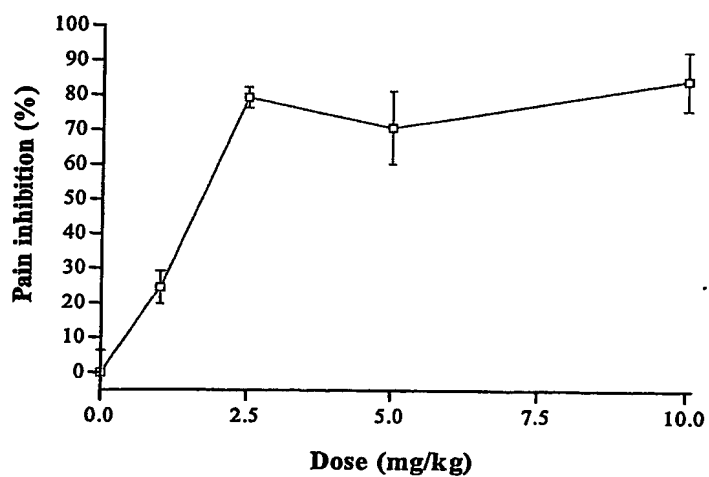


FIG 20 - Dose response curves for water extract ($\bar{x} \pm SE$, n=4).

12/35

**FIG 21 - Preparative gel permeation column.****FIG 22 - Dose response curve for TSK-4a ($\bar{x} \pm SE$, n=3).**

13/35

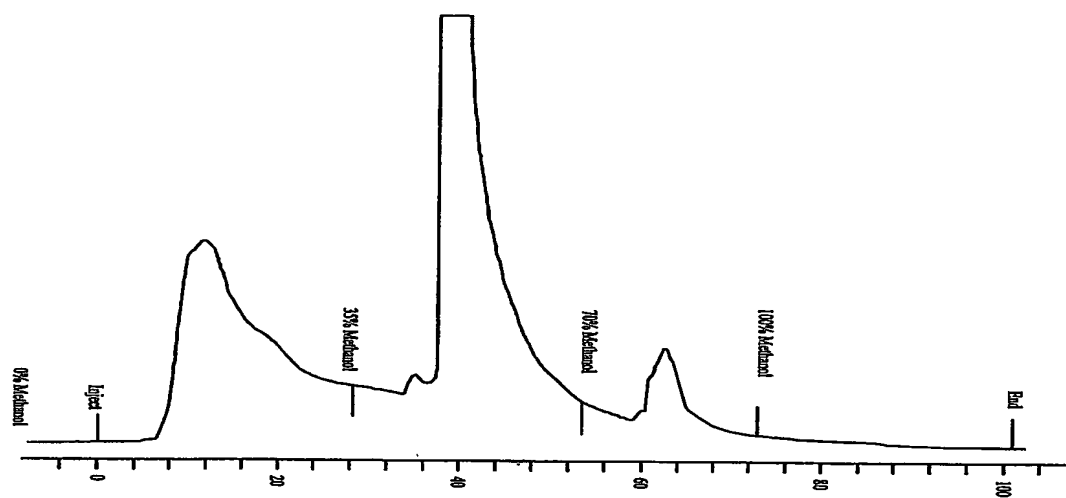


FIG 23 - C18 separation of TSK-4a.

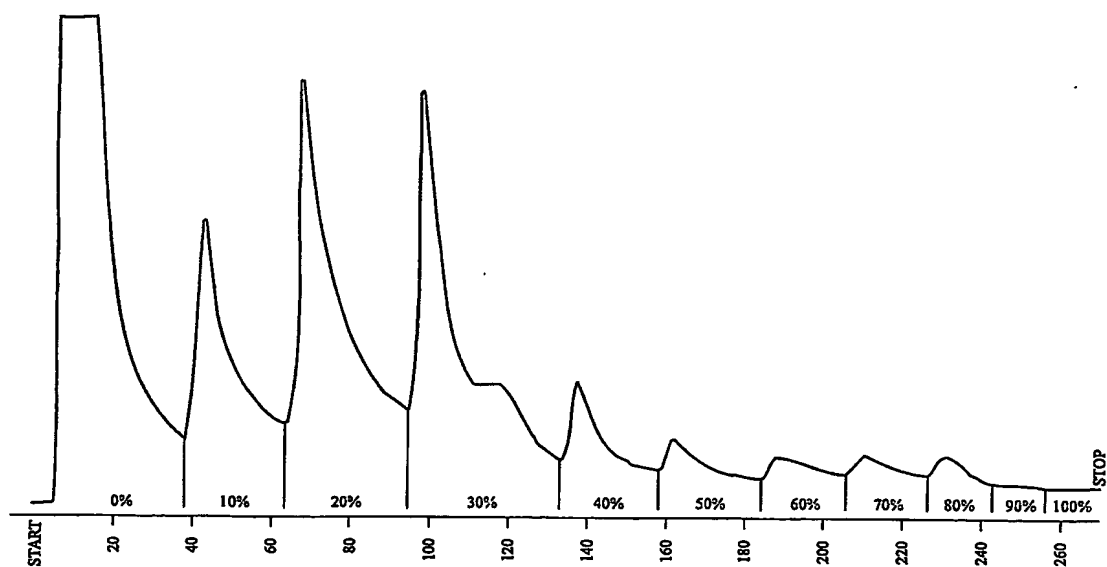
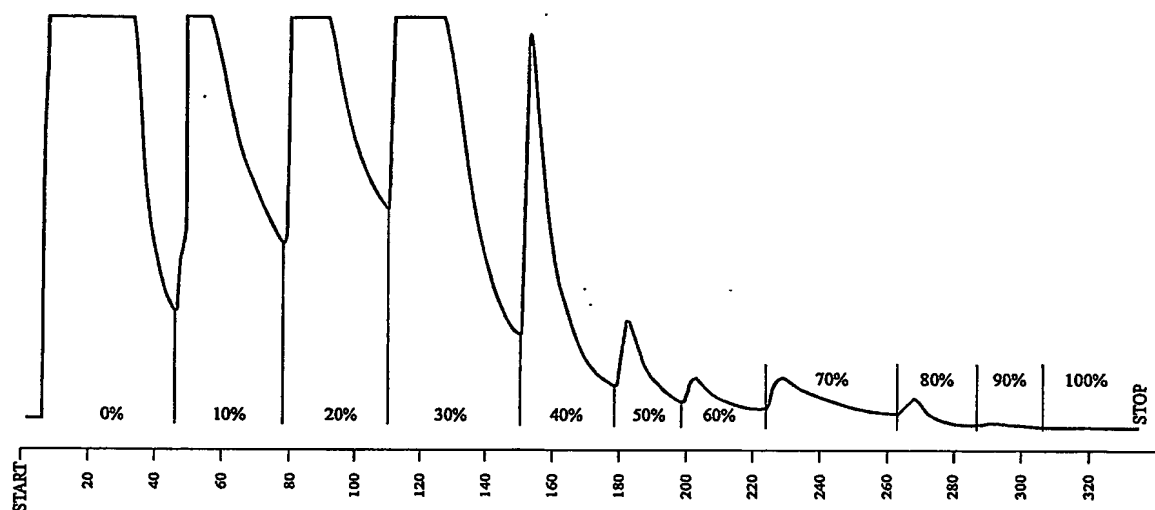
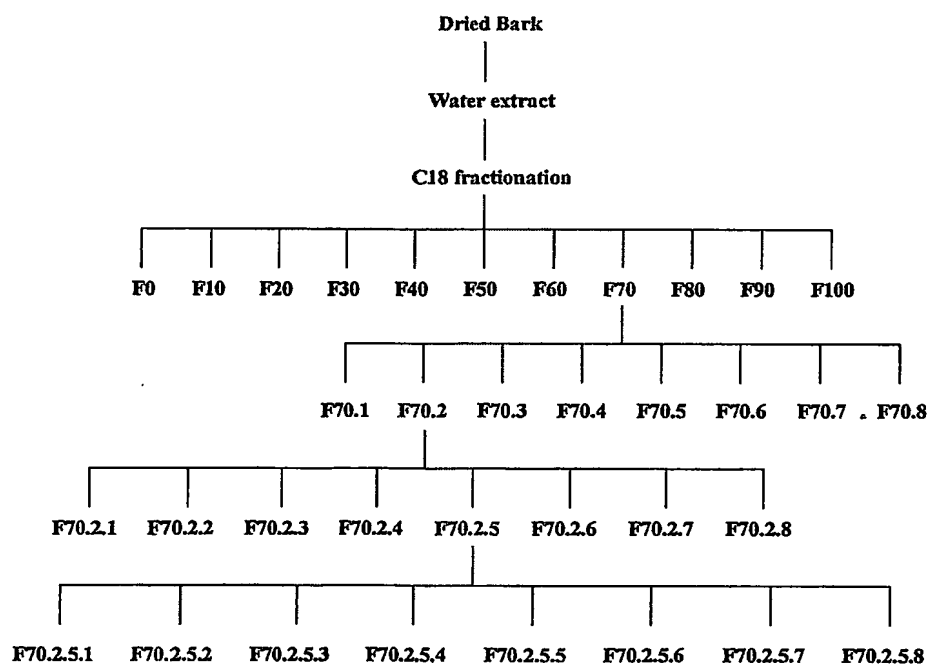


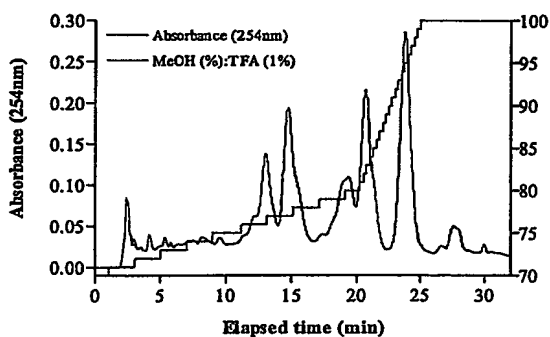
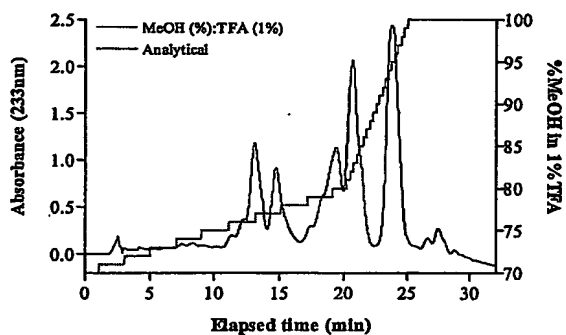
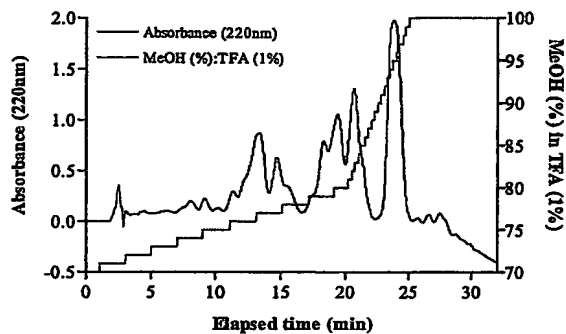
FIG 24 - C18 preparative separation of TSK-4a.

14/35

**FIG 25 - Preparative C18 chromatogram of H₂O extract****FIG 26 - Outline of numbering system compound F70.2.5.2.**

15/35

Analytical separations



Preparatory separations

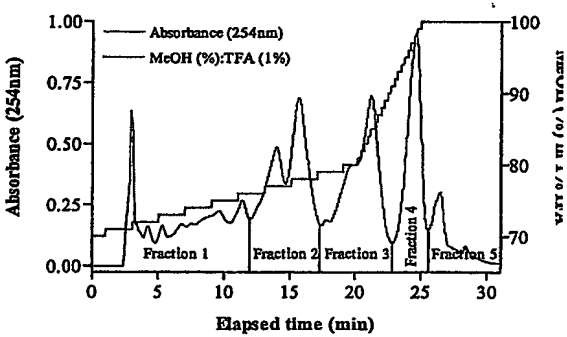
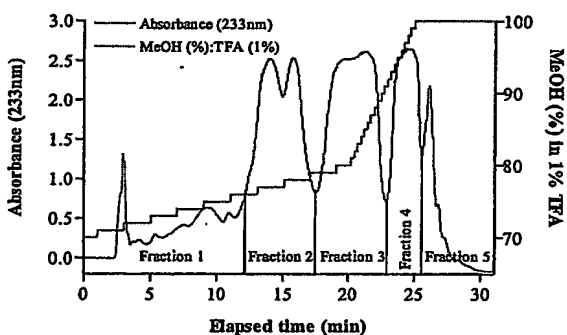
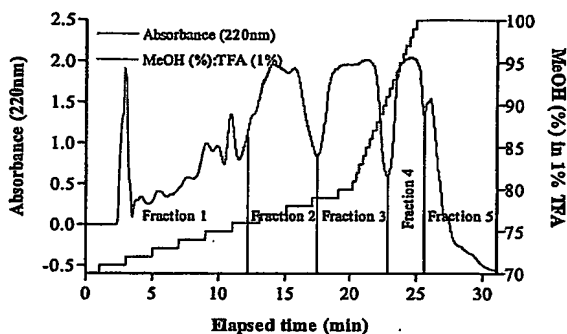
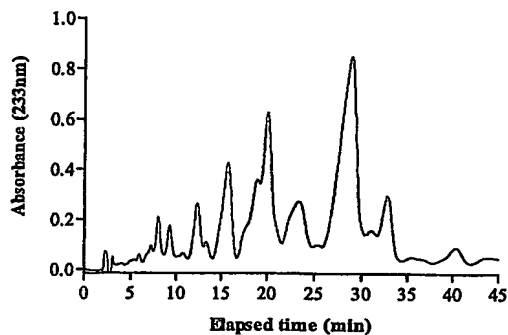


FIG 27 - Separation of fraction eluting at 70% MeOH (F70).

16/35

Analytical separations



Preparatory separations

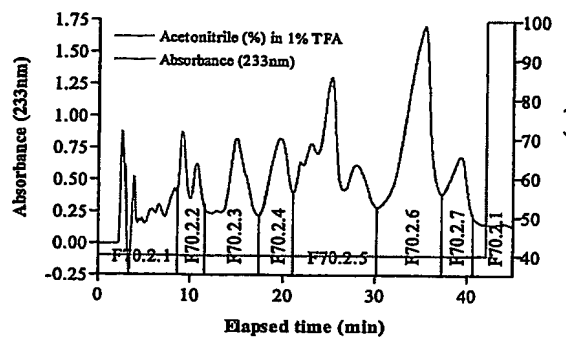


FIG 28 - Separation of fraction F70.2 (40%MeCN in 1%TFA).

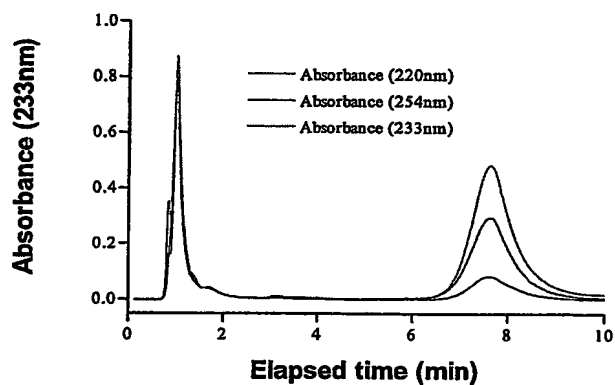


FIG 29 - Chromatogram of F70.2.6.

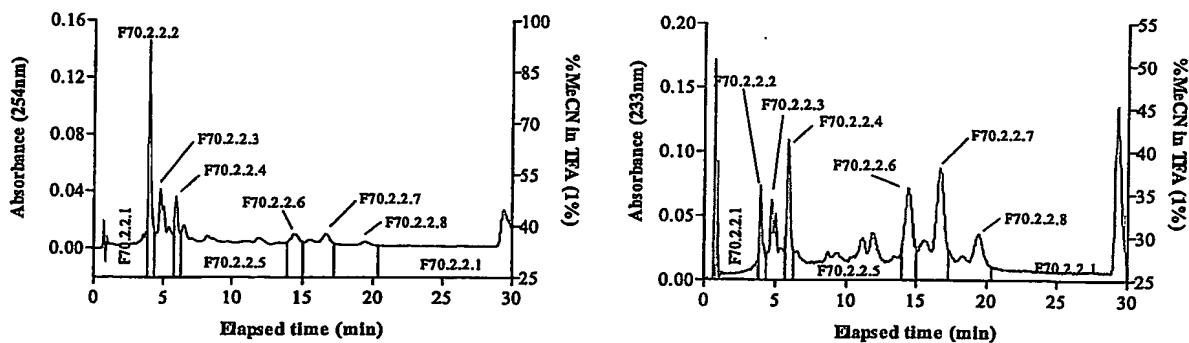


FIG 30 - Separation of fraction F70.2.2 at 254nm (left) and 233nm (right).

17/35

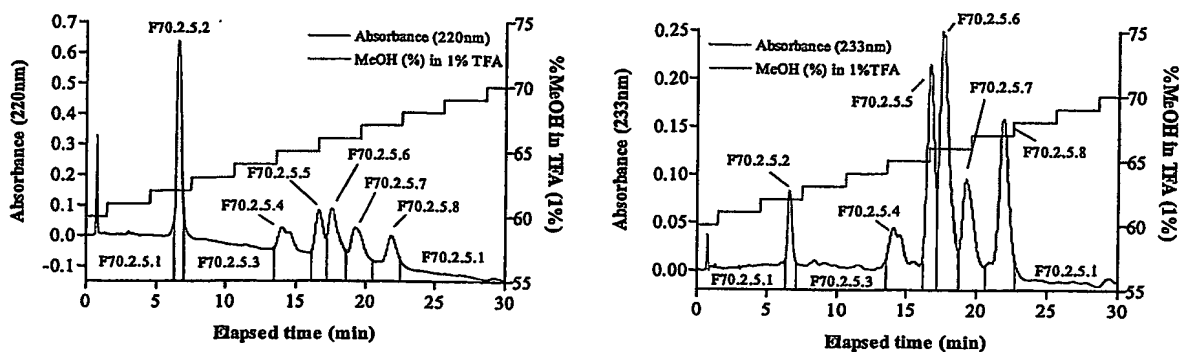
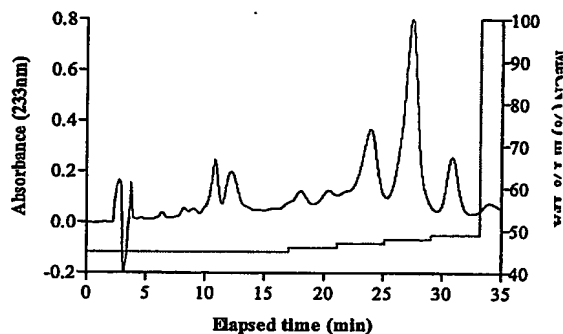


FIG 31 - Separation of fraction F70.2.5 at 220nm (left) and 233nm (right).

Analytical separations



Preparatory separations

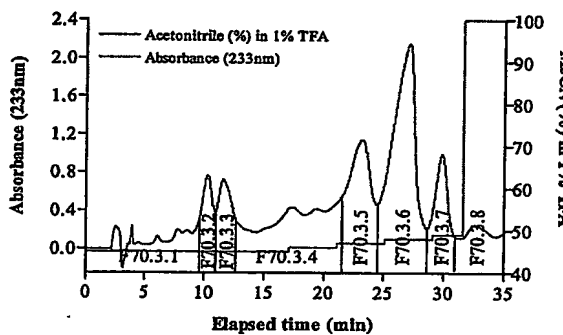
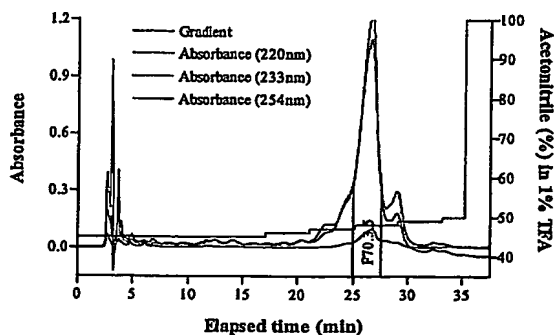


FIG 32 - Separation of fraction F70.3.

Fraction F70.3.5



Fraction F70.3.7

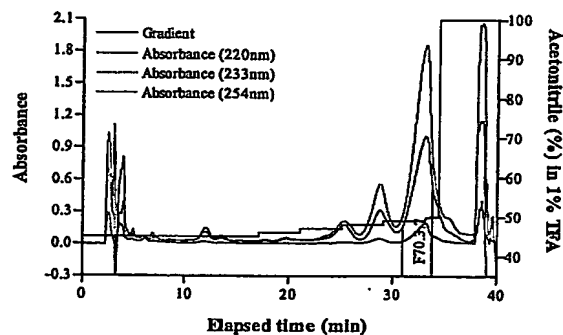
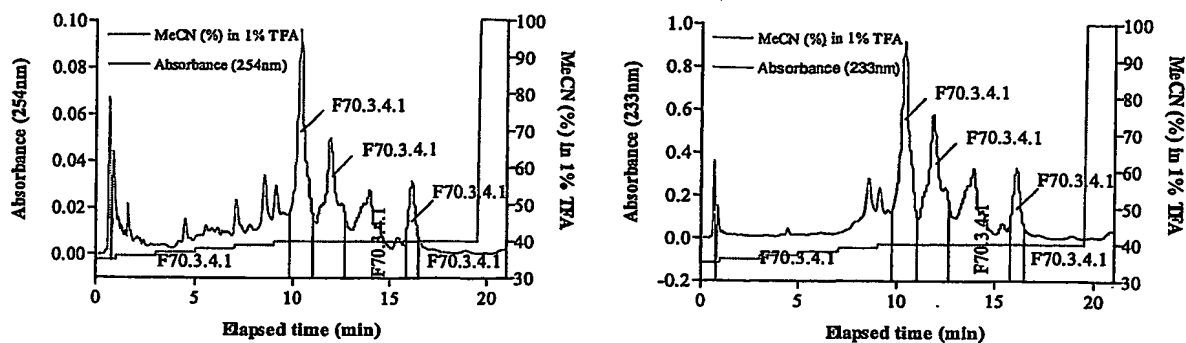
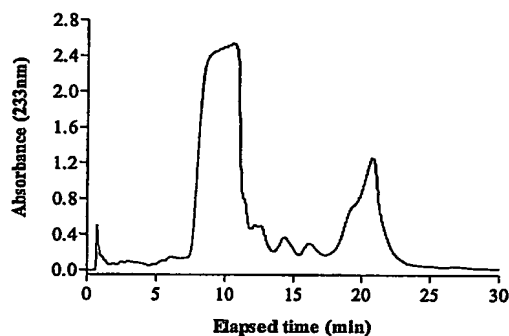


FIG 33 - Chromatograms of F70.3.5 and F70.3.7.

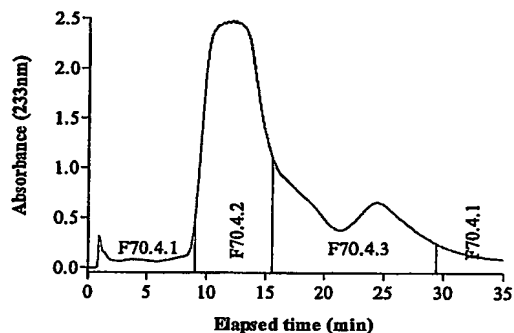
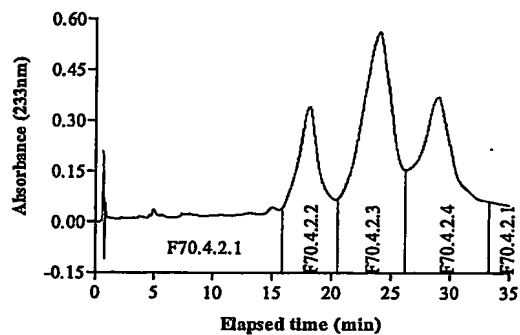
18/35

**FIG 34 - Analytical separation of fraction F70.3.4 at 254 and 233nm.**

Analytical separation



Preparative separation

**FIG 35 - Separation of F70.4.****FIG 36 - Separation of F70.4.2.**

19/35

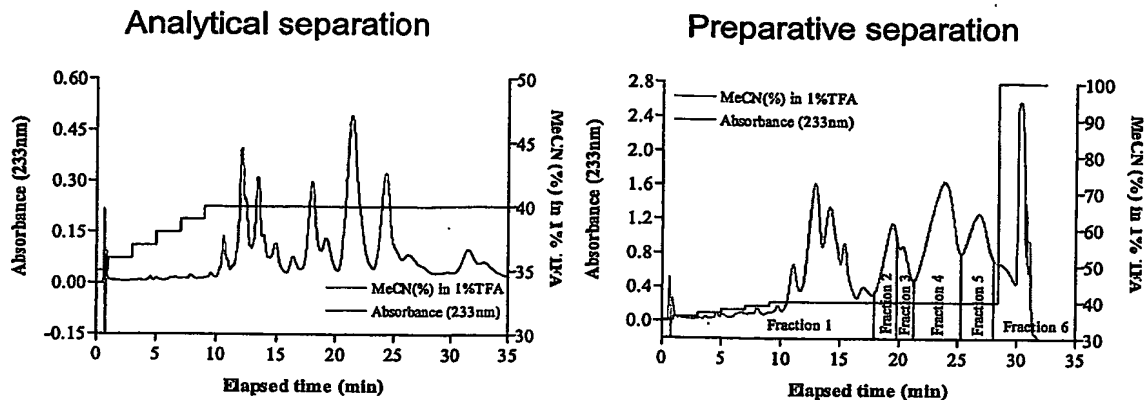


FIG 37 – Analytical separation (left) and preparative separation (right) of F70.4.3.

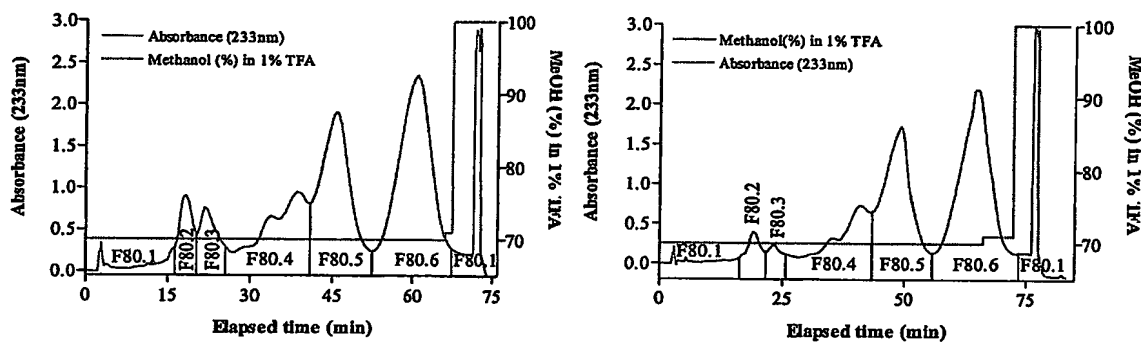


FIG 38 - Preparative chromatograms showing loss of peaks F80.2 & F80.3.

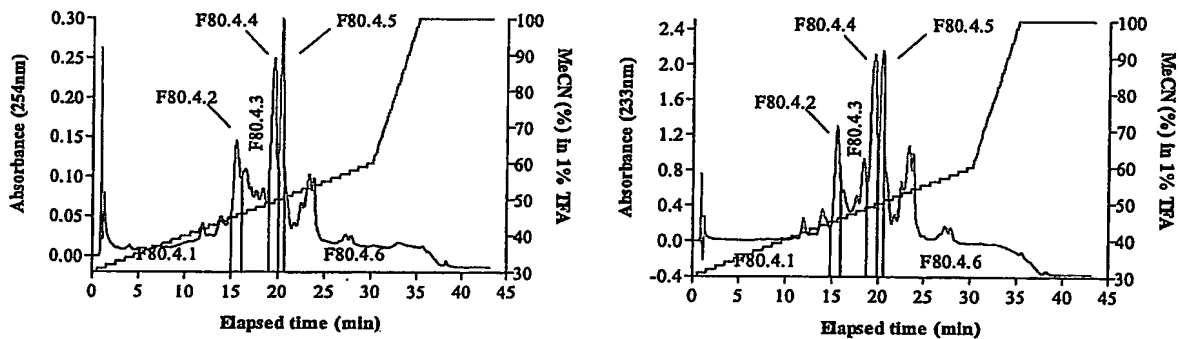


FIG 39 - Preparative chromatograms of F80.4.

20/35

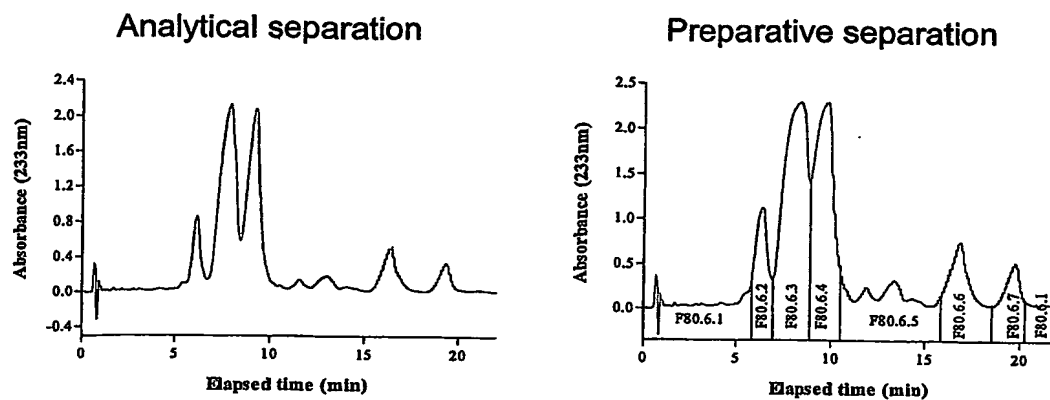


FIG 40 - Separation of fraction F80.6 using a phenyl reverse phase column with the analytical separation (left) and the preparative separation (right).

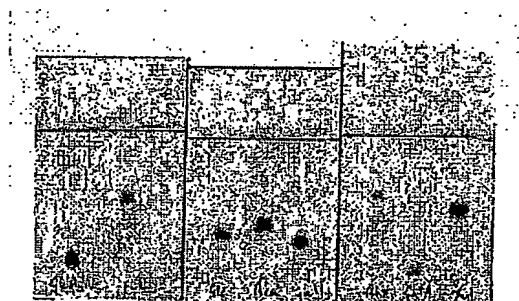


FIG 41 - Standard sugars used for TLC

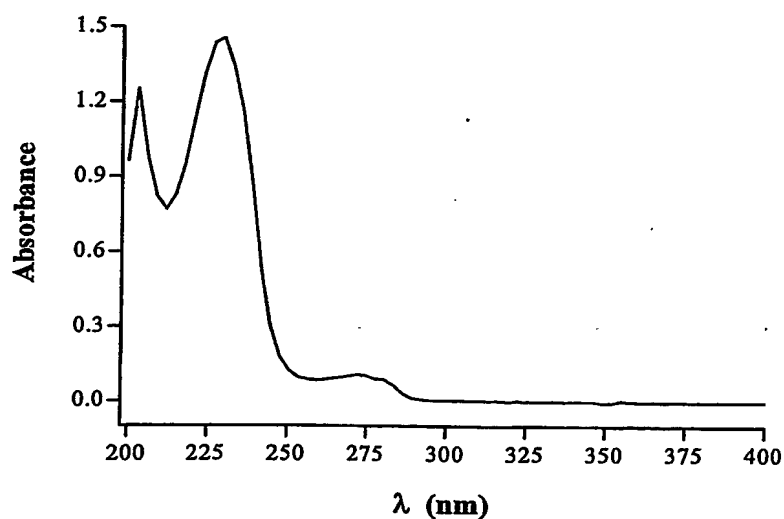
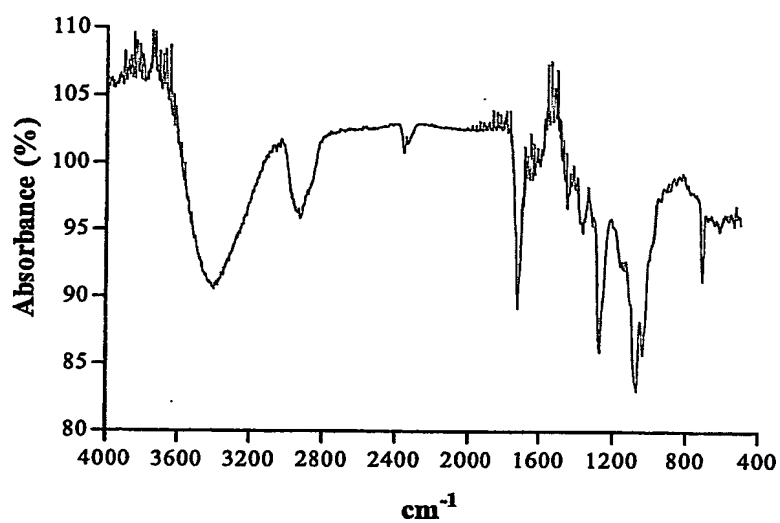
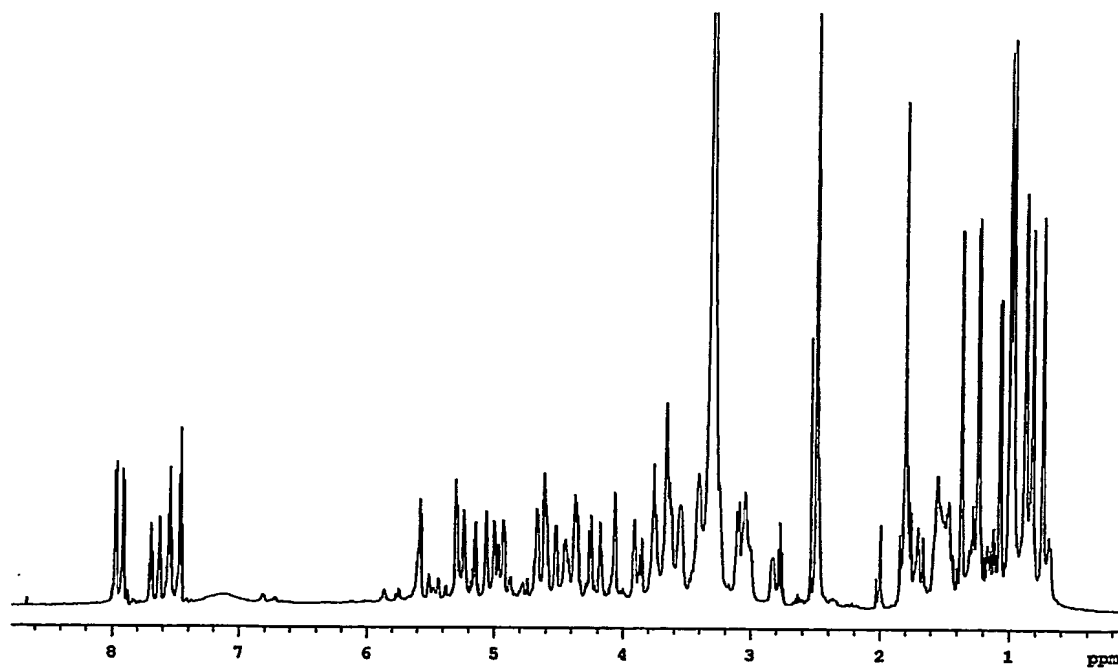


FIG 42 - UV spectrum of F70.3.6

21/35**FIG 43 - FTIR spectrum of F70.3.6****FIG 44 - ¹H-NMR for compound F70.3.6**

22/35

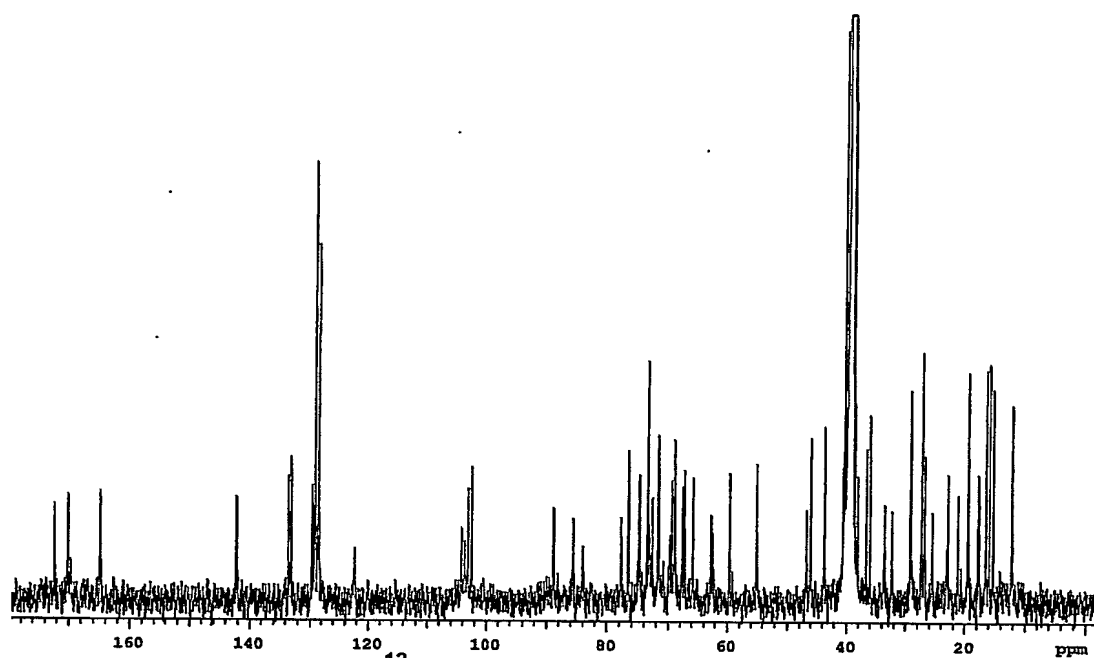
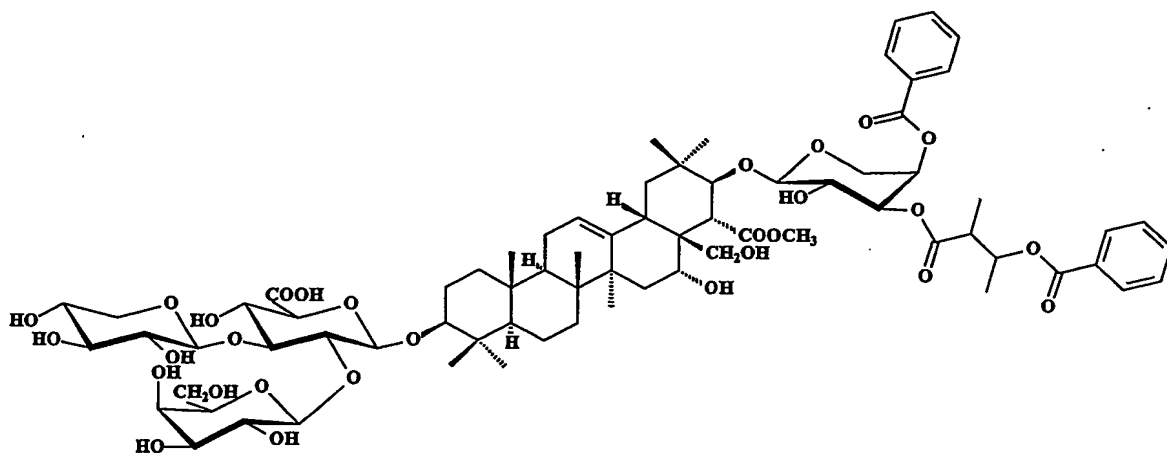
FIG 45 - ^{13}C -NMR for compound F70.3.6

FIG 46 - The complete assignment of F70.3.6
(3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-21-O-[3-(3-benzoyl-2-methylbutanoyl)-4-benzoyl- α -L-arabinopyranosyl]-22-O-acetyl barringtogenol C)

23/35

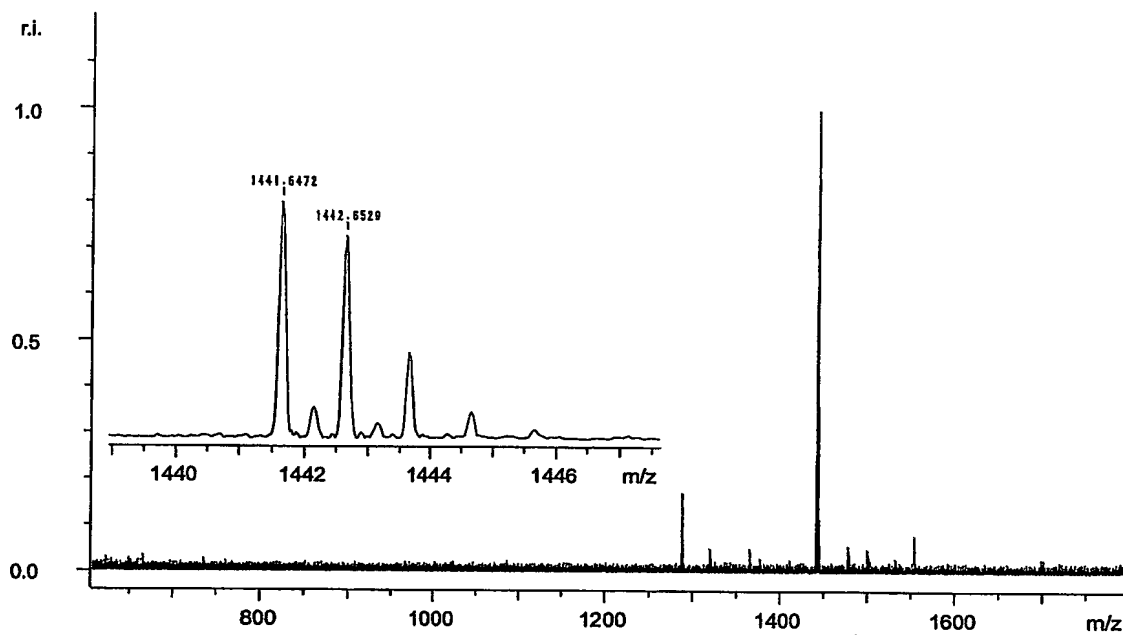


FIG 47 - Negative ion HR-ESMS of F70.3.6 (insets show detail of molecular ion)

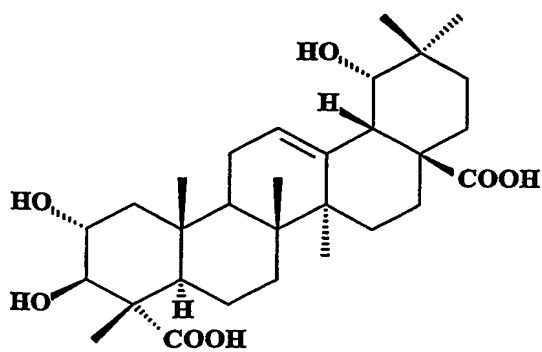
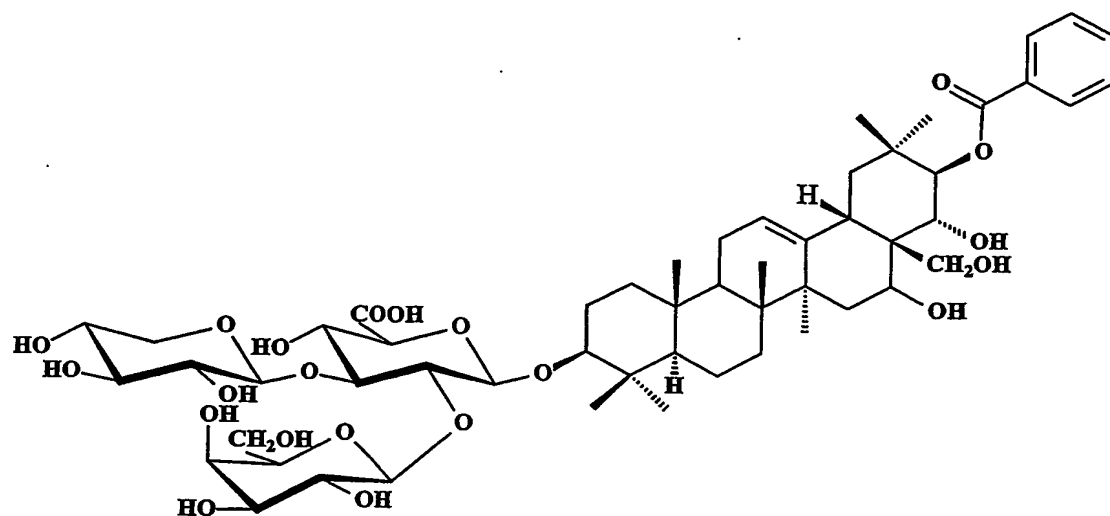


FIG 48 - Compound F70.2.5.2
(2 α , 3 β , 19 α -trihydroxy-olean-12-ene-23, 28-dioic acid)

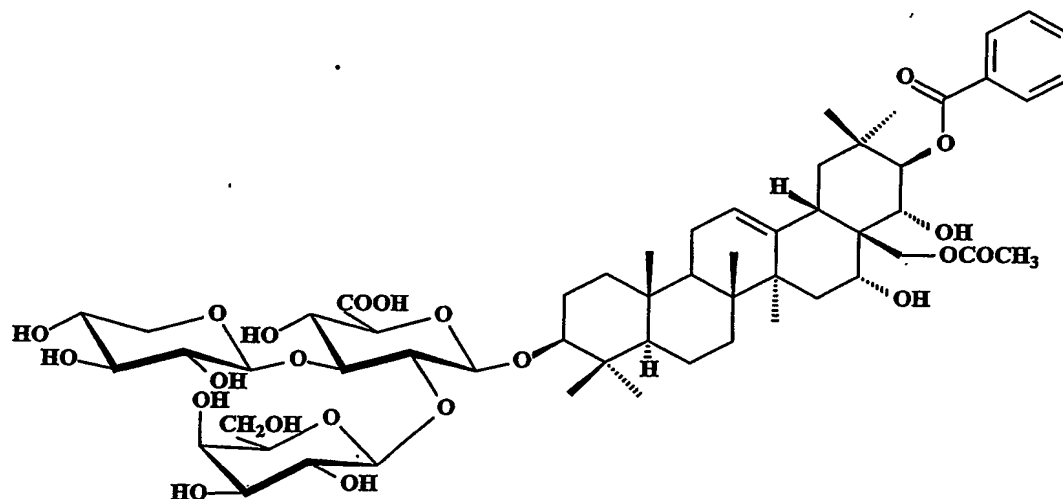
24/35



2

FIG 49 - Compound F70.2.3.

(3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-21-O-benzoyl barringtogenol C)

**FIG 50 - Compound F70.3.2**

3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-21-O-benzoyl-28-O-acetyl barringtogenol C)

25/35

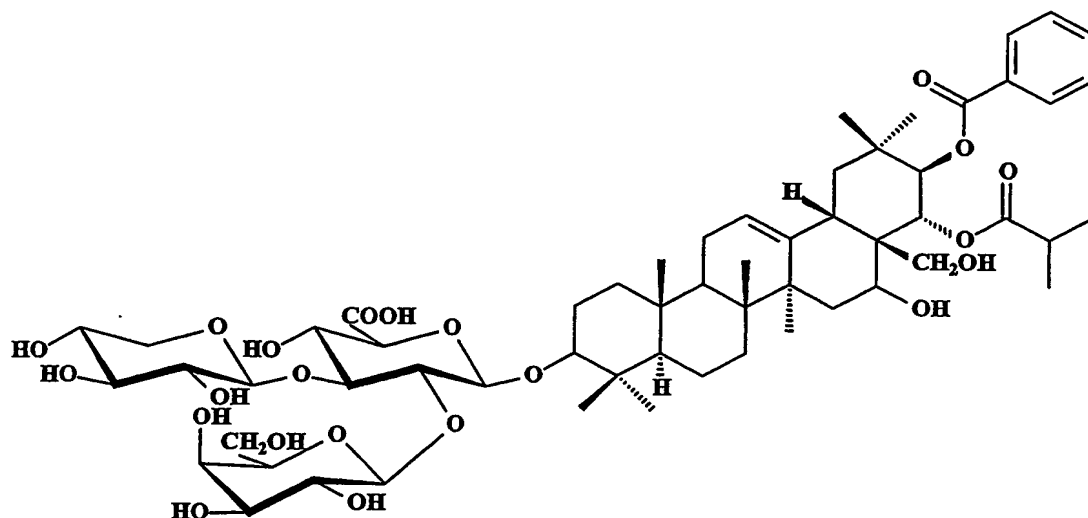


FIG 51 - Compound F70.3.4.2
(3-O-β-D-xylopyranosyl(1→3)-[β-D-galactopyranosyl(1→2)]-β-D-glucuronopyranosyl-21-O-benzoyl-22-O-isobutyryl barringtogenol C)

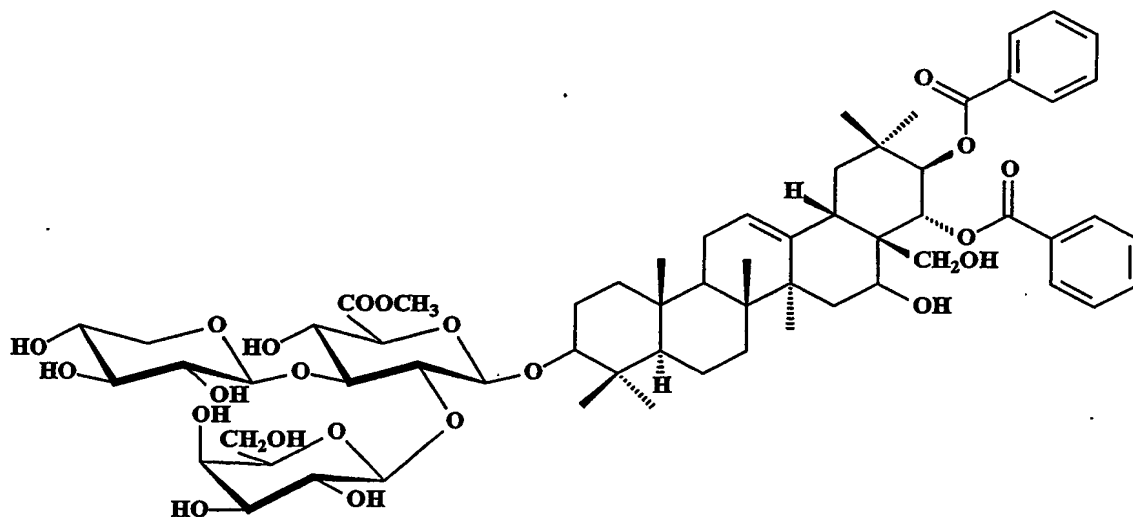


FIG 52 - Compounds F70.4.3.5.2/F80.6.7
(3-O-β-D-xylopyranosyl(1→3)-[β-D-galactopyranosyl(1→2)]-β-D-methylglucuronopyranosyl-21,22-O-dibenzoyl barringtogenol C)

26/35

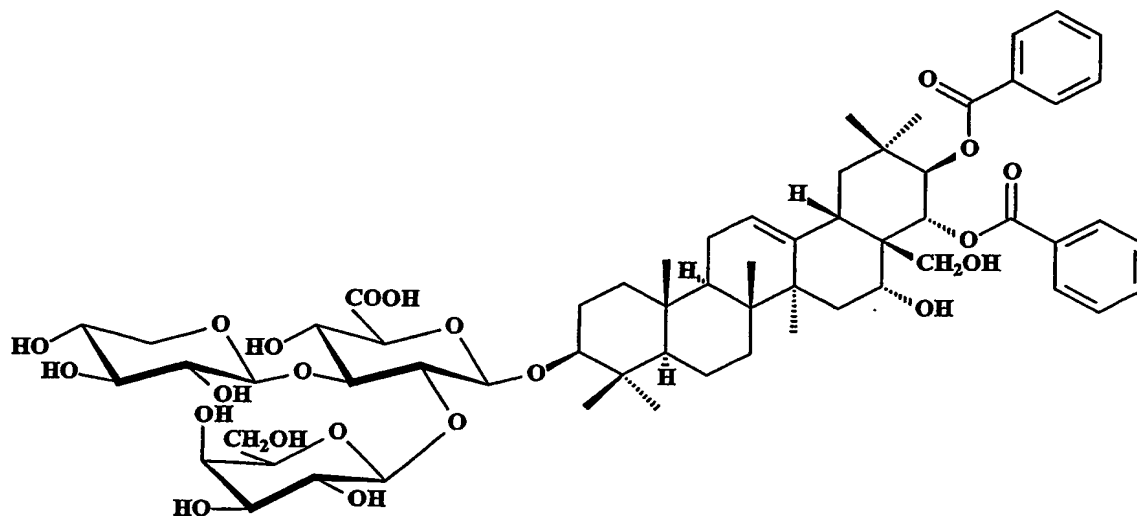


FIG 53 - Compound F80.6.4/F70.4.2.4.2
(3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-21, 22-O-dibenzoyl barringtogenol C

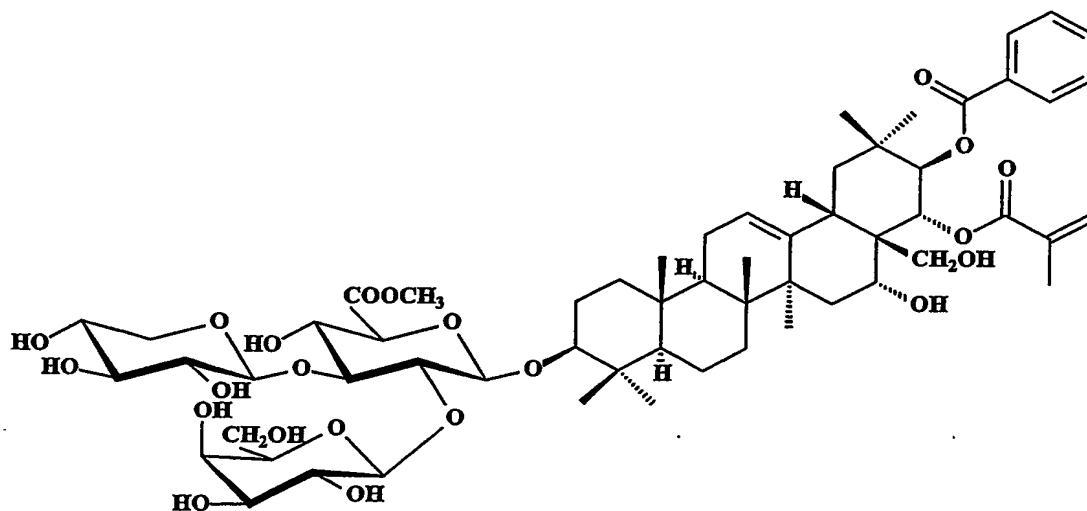


FIG 54 - Compound F70.4.3.4.2/F80.6.6
(3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-methylglucuronopyranosyl-21-O-benzoyl-22-O-tigloyl barringtogenol C)

27/35

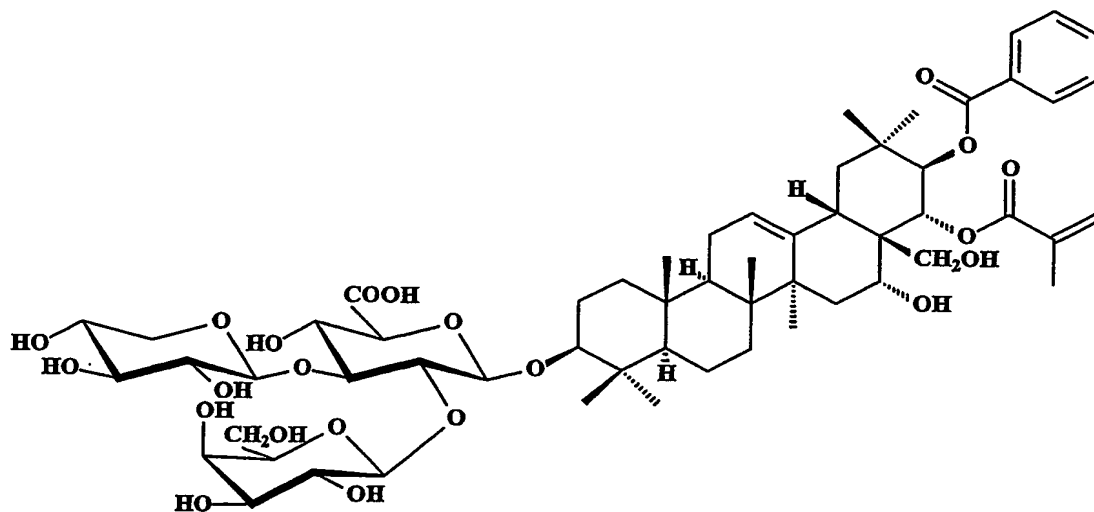


FIG 55 - Compound F70.4.2.3/F80.6.3
(3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-21-O-benzoyl-22-O-tigloyl barringtonenol C)

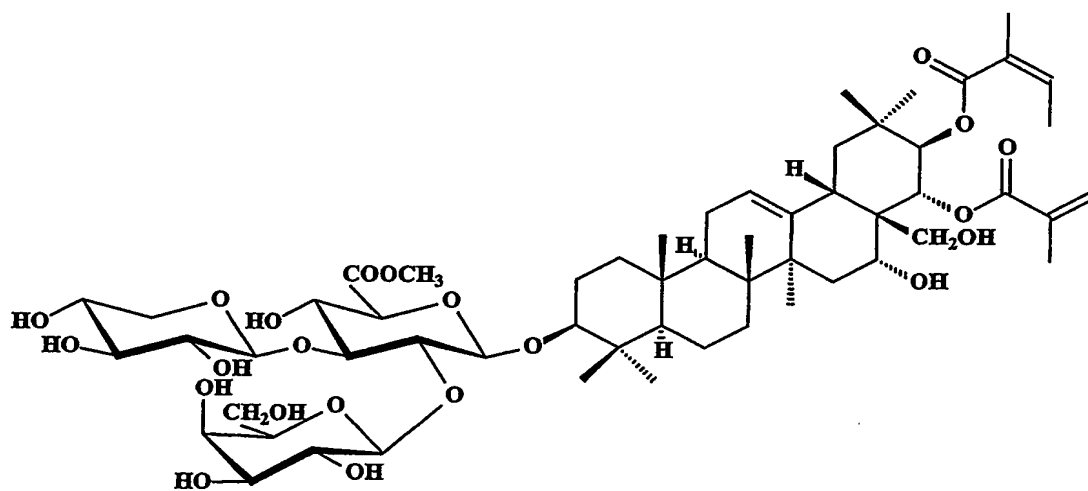


FIG 56 - Compound F70.4.3.2.2
(3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-methylglucuronopyranosyl-21,22-O-tigloyl barringtonenol C)

28/35

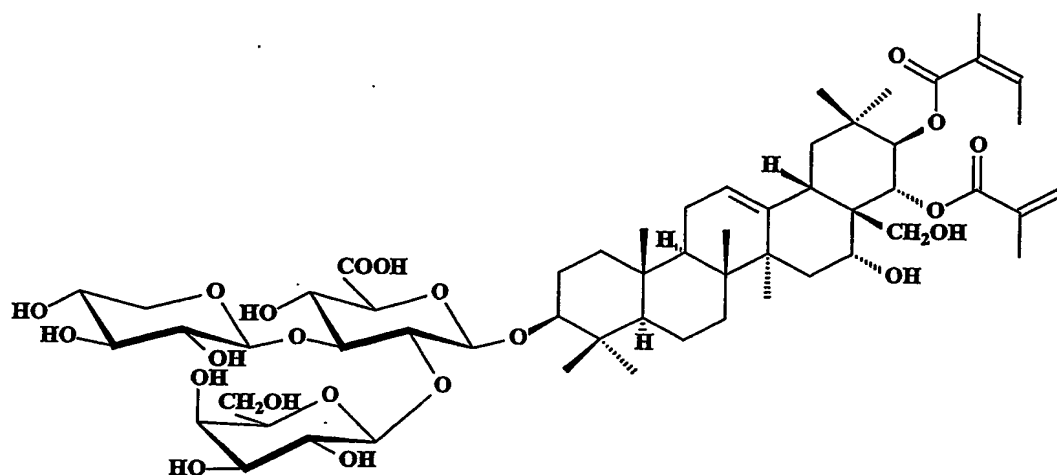


FIG 57 - Compound F80.6.2
(3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-21,22-O-tigloyl barringtogenol C)

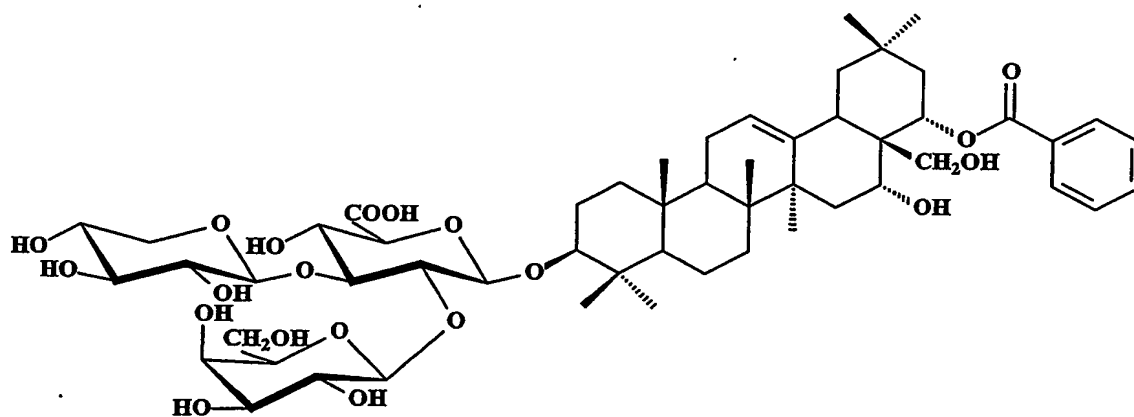


FIG 58 - Compound F70.3.3.2.2b
(3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-22-O-benzoyl barringtogenol C)

29/35

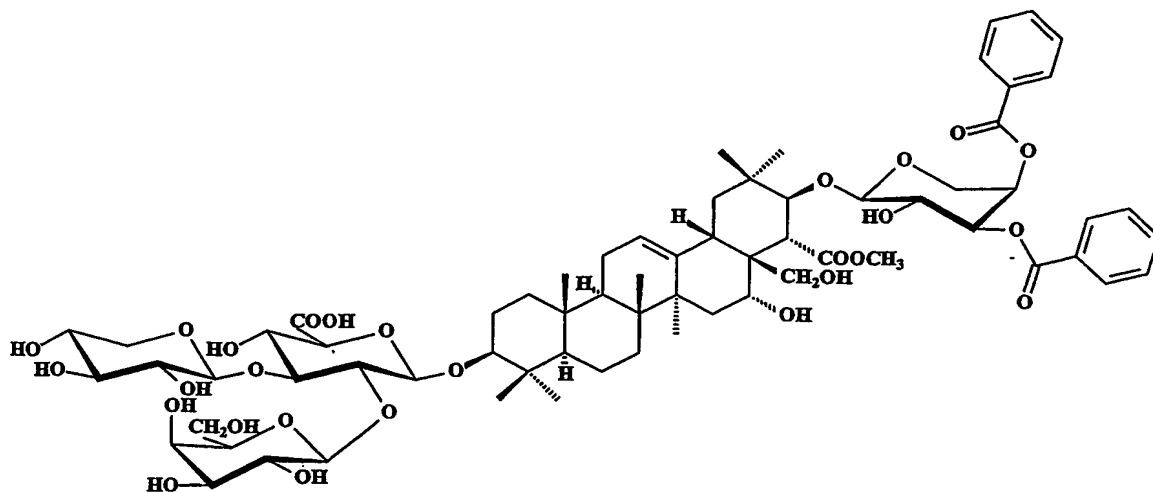


FIG 59 - Compound F70.2.6.2
 (3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-21-O-[3,4-dibenzoyl- γ -L-arabinopyranosyl]-22-O-acetyl barringtogenol C)

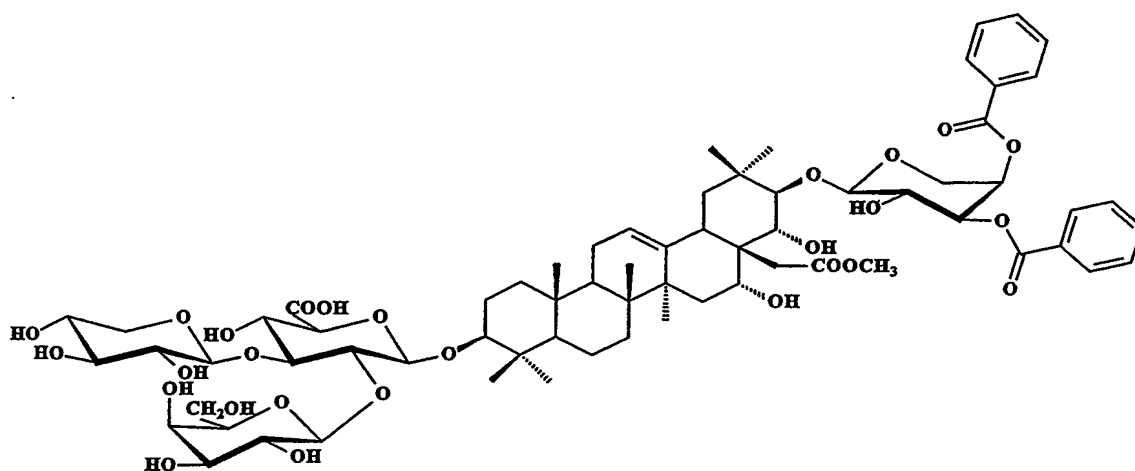
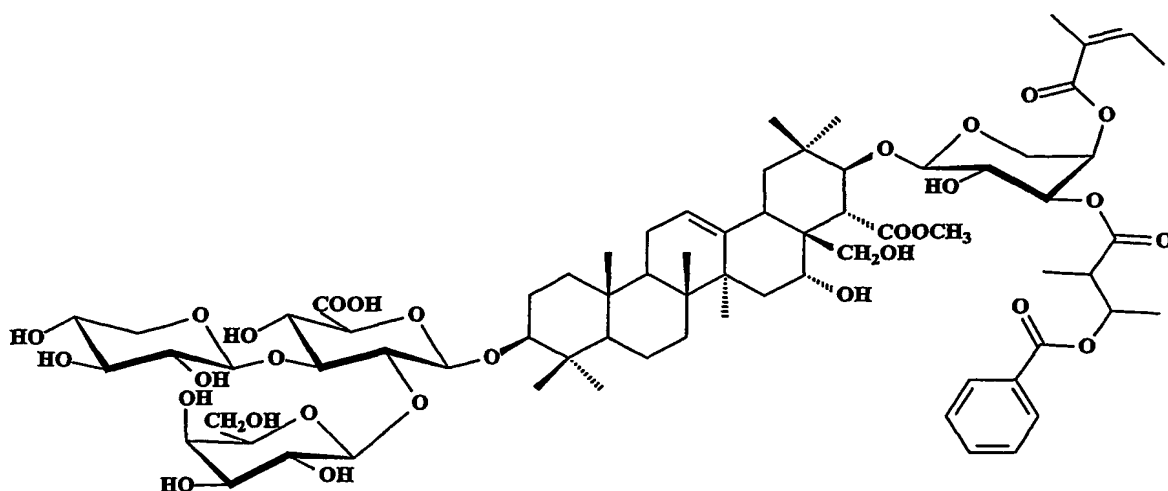
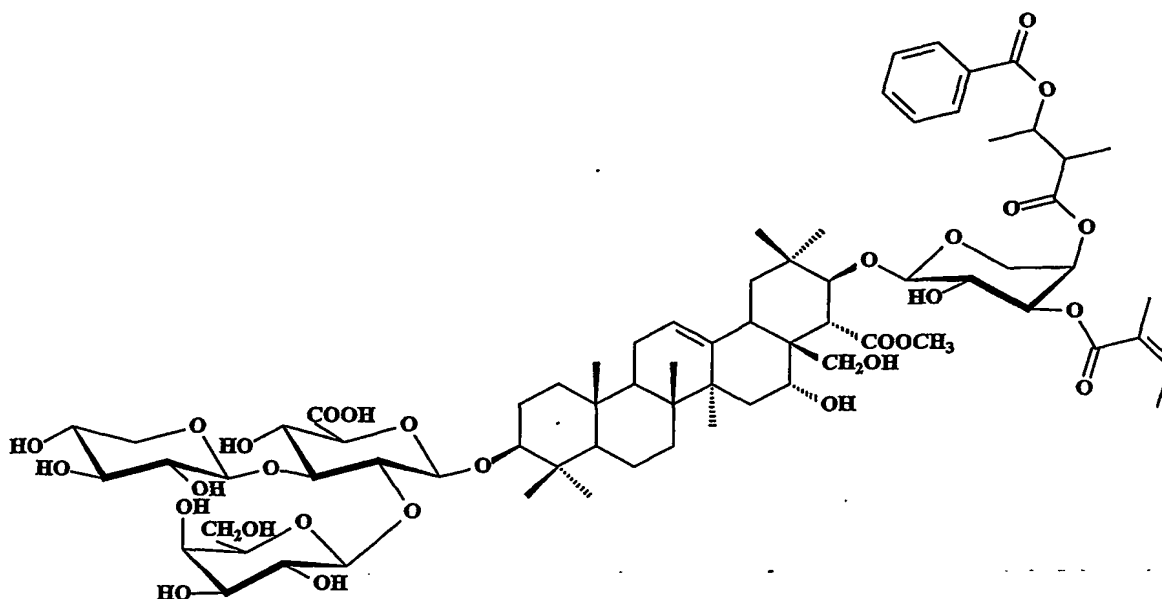


FIG 60 - Compound F70.3.4.5
 (3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-21-O-[3,4-dibenzoyl- α -L-arabinopyranosyl]-28-O-acetyl barringtogenol C)

30/35

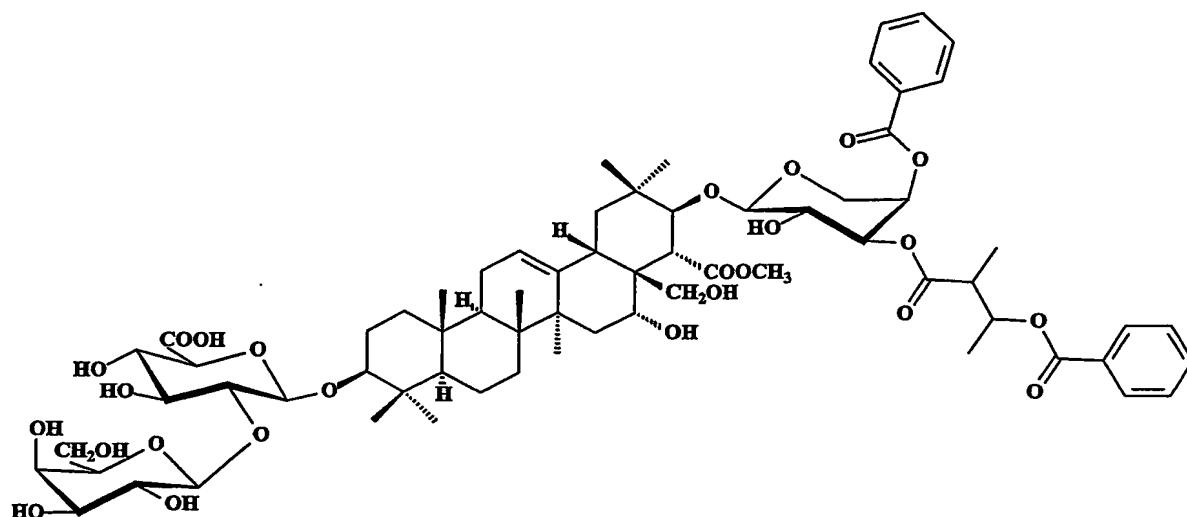
**FIG 61 - Compound F70.3.5a**

(3-O-β-D-xylopyranosyl(1→3)-[β-D-galactopyranosyl(1→2)]-β-D-glucuronopyranosyl-21-O-[3-(3-benzoyl-2-methylbutyryl)-4-tigloyl-α-L-arabinopyranosyl]-22-O-acetyl barringtogenol C)

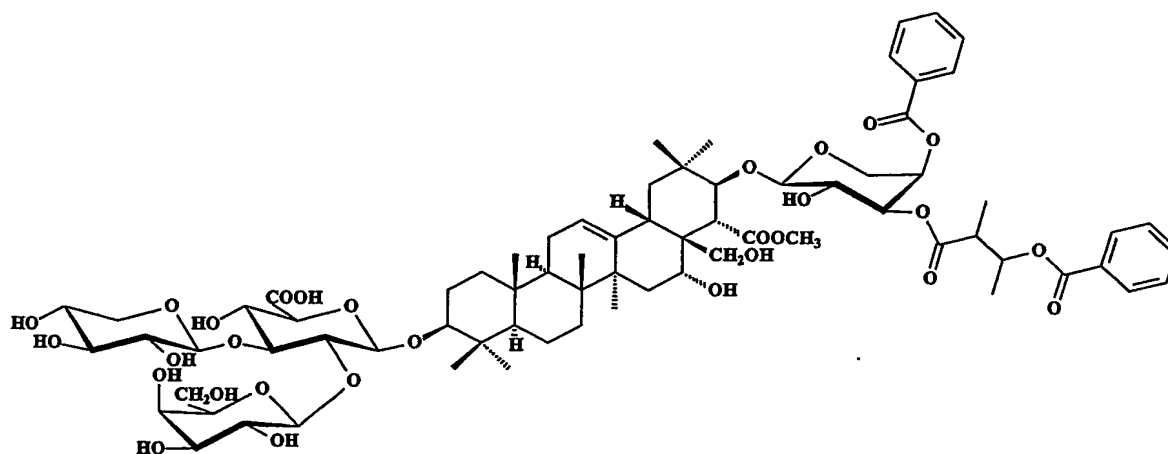
**FIG 62 - Compound F70.3.5b**

(3-O-β-D-xylopyranosyl(1→3)-[β-D-galactopyranosyl(1→2)]-β-D-glucuronopyranosyl-21-O-[3-tigloyl-4-(3-benzoyl-2-methylbutyryl)-α-L-arabinopyranosyl]-22-O-acetyl barringtogenol C)

31/35

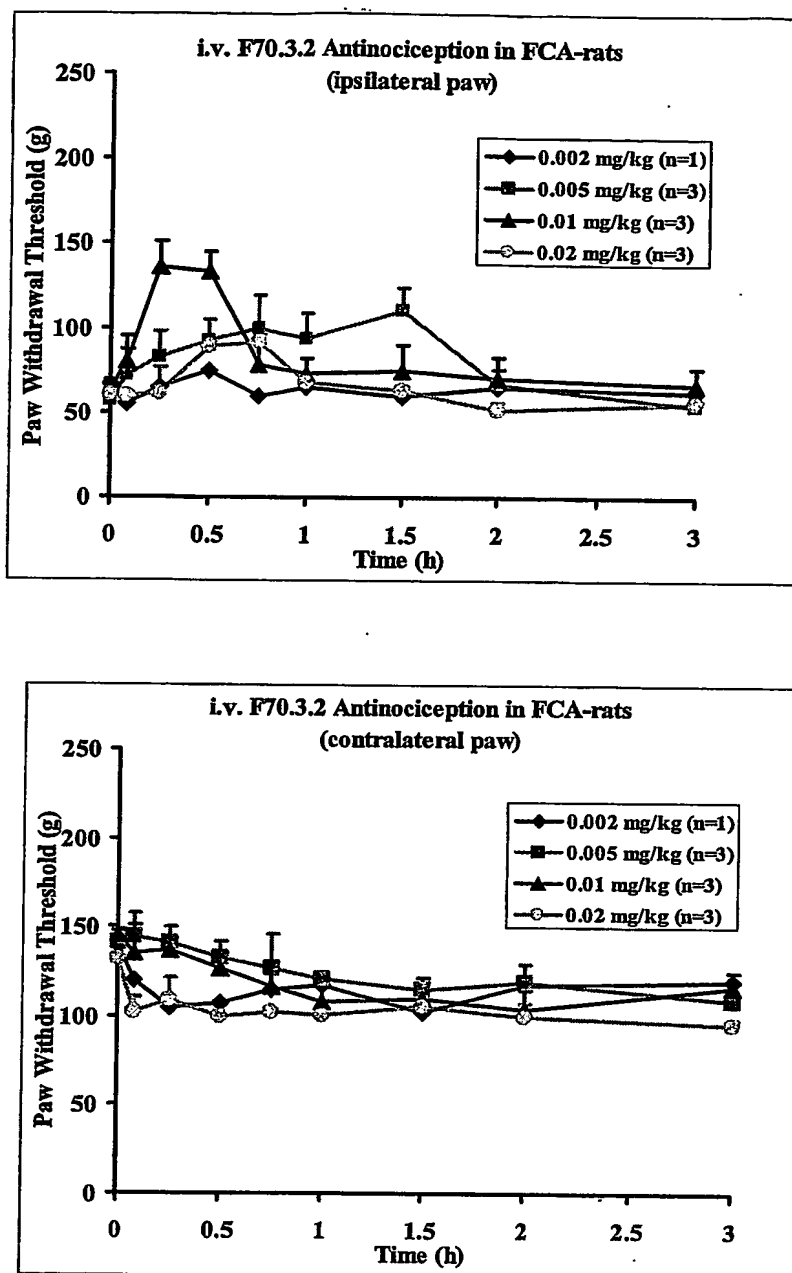
**FIG 63 - Compound F70.3.7.2**

(3-O- β -D-galactopyranosyl(1 \rightarrow 2)- β -D-glucuronopyranosyl-21-O-[3-(3-benzoyl-2-methylbutyryl)-4-benzoyl- α -L-arabinopyranosyl]-22-O-acetyl barringtogenol C)

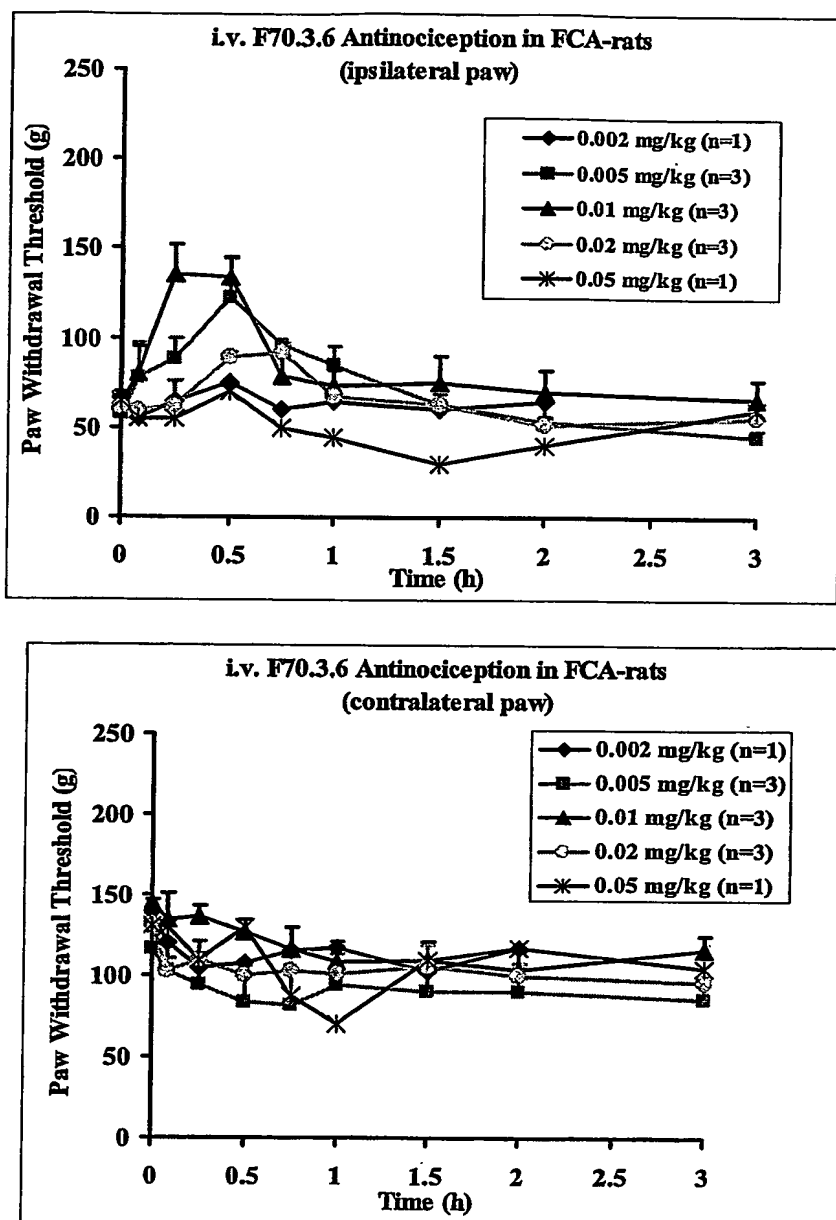
**FIG 64 - Compound F80.4.5.2/F80.5.2**

(3-O- β -D-xylopyranosyl(1 \rightarrow 3)-[β -D-galactopyranosyl(1 \rightarrow 2)]- β -D-glucuronopyranosyl-21-O-[3-(3-benzoyl-2-methylbutyryl)-4-benzoyl- α -L-arabinopyranosyl]-28-O-acetyl barringtogenol C)

32/35



33/35



34/35

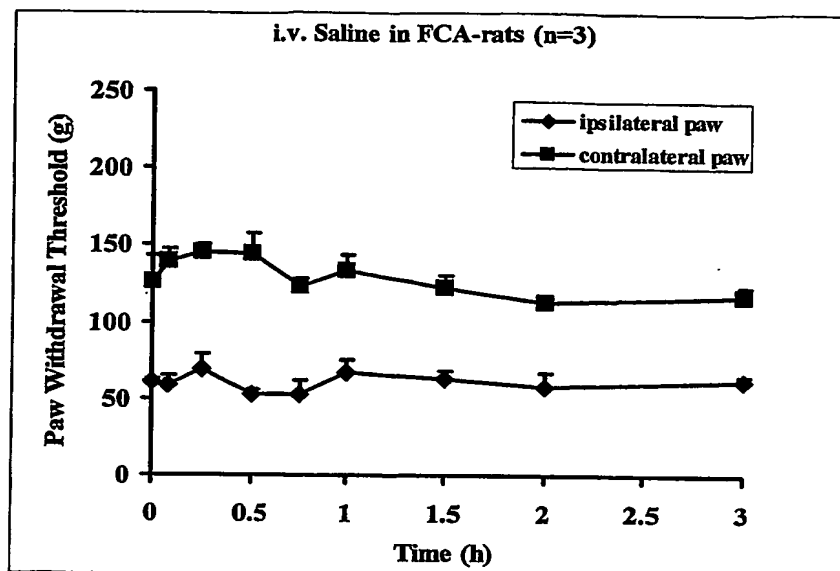


FIG. 67: is the mean (\pm SEM) paw withdrawal threshold versus time curve for the ipsilateral (inflamed) and the contralateral (non-inflamed) hindpaw in FCA-treated adult male Sprague-Dawley rats (n = 3) that received a single i.v. bolus of saline.

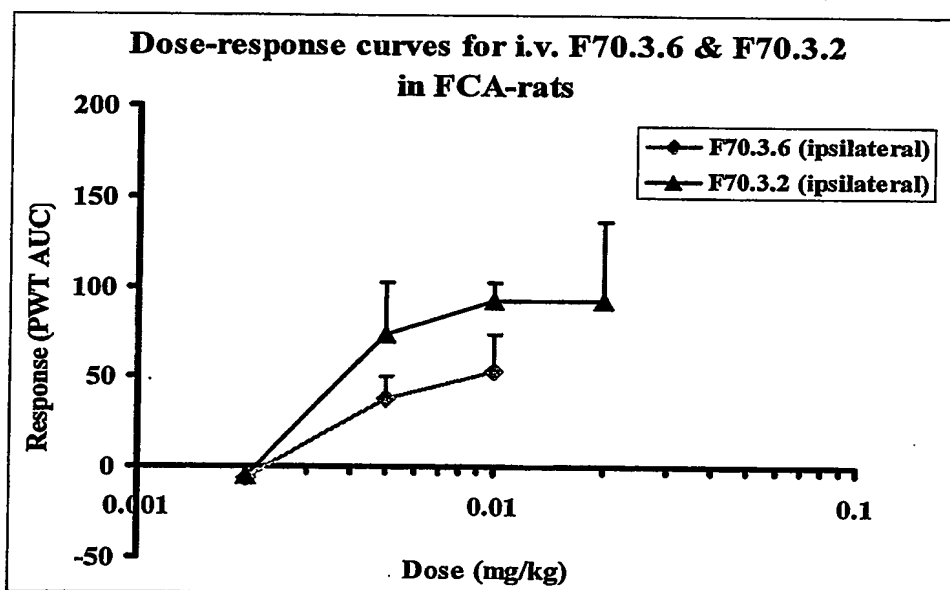


FIG. 68: Mean (\pm SEM) dose-response curves for the antinociceptive effects of i.v. bolus doses of F70.3.2 and F70.3.6 in the ipsilateral hindpaws of FCA-rats.

35/35

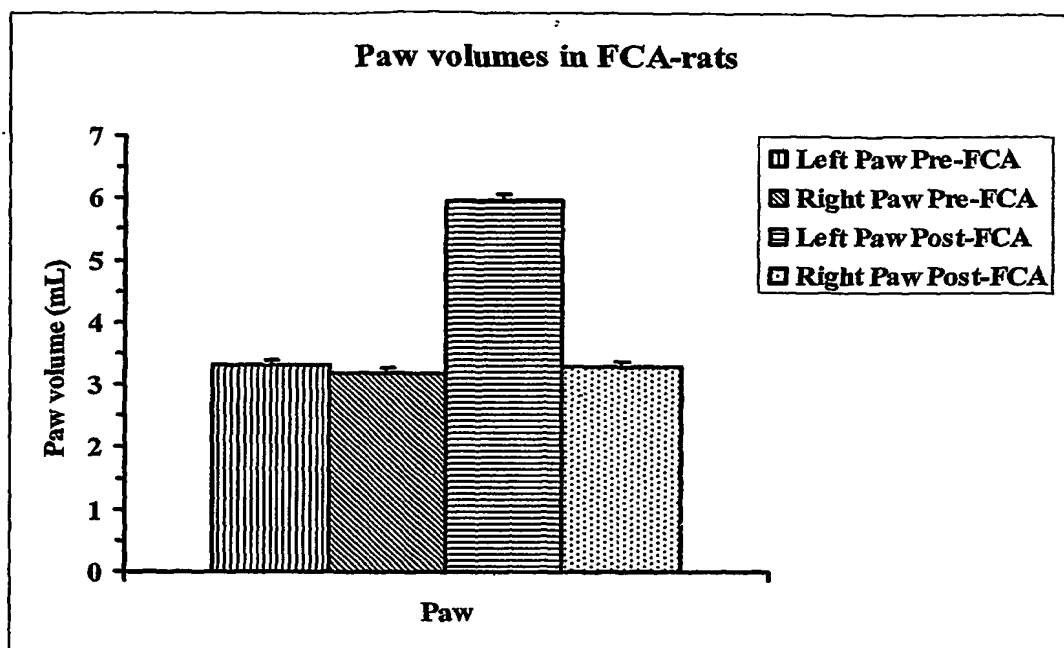


FIG. 69 is a graph of the paw volume pre and post FCA treatment.